EVALUATION OF WATER QUALITY OF RESERVOIRS FROM CÁVADO RIVER'S HYDROGRAPHIC BASIN

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

MSc

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Resumo

As albufeiras são massas de água fortemente modificadas, formadas, normalmente, em consequência da construção de barragens. A interrupção do curso natural do rio tem alterações significativas no ecossistema e na qualidade da água. As albufeiras são ecossistemas sujeitos à acumulação de nutrientes e a elevadas variações no nível da água, estando assim mais sujeitas a processos de eutrofização e perturbações na estabilidade das comunidades biológicas. Com o aumento da dependência das populações humanas no abastecimento de água fornecido pelas albufeiras, torna-se cada vez mais importante criar ferramentas e metodologias para avaliar a qualidade da água e a estabilidade dos ecossistemas aquáticos. Em resposta a esta necessidade têm vindo a ser criados documentos que permitem a avaliação da qualidade da água em albufeiras, tais como a Diretiva Quadro da Água (DQA). A DQA propõe a utilização de elementos físicos e químicos, hidromorfológicos e biológicos para avaliar a qualidade da água. O zooplâncton é um elemento biológico de todos os ecossistemas aquáticos, apresentando um papel muito importante nas teias tróficas, e que apresenta elevada sensibilidade para alterações que ocorram no ecossistema. Apesar disto, a DQA não inclui o zooplâncton como um dos elementos biológicos a utilizar na classificação das massas de água. Assim, o principal objetivo deste projeto foi avaliar a qualidade da massa de água em quatro albufeiras pertencentes à bacia hidrográfica do rio Cávado (Venda Nova, Alto Cávado, Alto Rabagão e Paradela), utilizando para isso elementos físicos, químicos e biológicos. Mensalmente (Março a Novembro de 2014) foram recolhidas amostras de água e de zooplâncton em cada albufeira. Os parâmetros físicos e químicos avaliados foram os propostos pela DQA. Relativamente ao zooplâncton este foi avaliado quanto à diversidade e abundância. Os resultados obtidos através dos parâmetros físicos e químicos permitiram classificar todas as albufeiras com “Bom Potencial Ecológico”. Contudo, a comparação destes resultados com a dinâmica do zooplâncton permitiu concluir que este elemento biológico é mais sensível a pequenas alterações no ecossistema. Assim, e olhando apenas para os parâmetros físicos e químicos propostos pela DQA para avaliar a qualidade da água, estes parecem ser altamente redutores para compreender todas as alterações que ocorrem nestes ecossistemas aquáticos.

Palavras-chave: Albufeiras, zooplâncton, Diretiva Quadro da Água, Índice do Estado Trófico, parâmetros físico-químicos, qualidade da água.

Abstract

Reservoirs are artificial lentic water bodies formed by the construction of dams. The sudden interruption of the normal flow of the river has major consequences to the ecosystem and the quality of the water. Reservoirs are more prone to nutrient accumulations and water-level fluctuations than natural lakes, thus being more susceptible to eutrophication and disturbance in the stability of the biological communities. With the increase in human dependence on reservoirs for water supply, there is an urgent need to create tools and methods to evaluate the quality of the water and the ecosystem stability. Concerning this, documents such as the Water Framework Directive (WFD), which provides guidelines to evaluate the ecological status of different types of water bodies, have been implemented to assess water quality in reservoirs. WFD proposes the use of physical, chemical, hydromorphological and biological elements to assess the quality of the water. Zooplankton is a biological element present in all aquatic ecosystems, plays a key role in the trophic webs and is highly sensible to changes in the environment. Despite this, WFD does not include zooplankton as one of the biological elements for the analysis of the water bodies. Therefore, the main aim of this study was to evaluate the water quality of four reservoirs (Venda Nova, Alto Cávado, Alto Rabagão and Paradela) belonging to Cávado River's hydrographic basin, using physical and chemical and biological indicators. To attain this objective, the reservoirs were sampled every month during nine months (between March and November of 2014). Samples of water and zooplankton were collected and brought to the laboratory for further analyses. Physical and chemical parameters evaluated were those proposed by WFD. Zooplankton samples were analysed concerning its composition, diversity and abundance. The physical and chemical data obtained allowed to classify all the reservoirs with "Good Ecological Potential". However, the comparison of these results with the dynamics observed in the zooplankton communities allowed to conclude that this biological element is more sensitive to small alterations in the ecosystem. Therefore, considering only the physical and chemical parameters proposed by WFD to evaluate water quality, they seem to be highly insufficient to understand all the alterations that occur in the aquatic ecosystems.

Keywords: Reservoirs, zooplankton, Water Framework Directive, Cávado River's basin, Trophic State Index, physical and chemical parameters, water quality

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List of Abbreviations

WFD	Water Framework Directive
ICOLD	International Commission on Large Dams
EDP	Energias de Portugal
TSI	Trophic State Index
GIG	Geographical Intercalibration Group
RH	Região Hidrográfica
PGRH	Plano de Gestão da Região Hidrográfica
POA	Plano de Ordenamento da Albufeira
TSS	Total Suspended Solids
CDOC	Organic Carbon Dissolved
BOD	Biochemical Oxygen Demand
PCA	Principal Components Analysis
CCA	Canonial Correspondence Analysis
GEP	Good Ecological Potential

Introduction

General Introduction

Water is one of the world's most important resources, being a vital support of the whole ecosystems. The existence of life on Earth requires the presence of water. Water is the only substance that occurs in appreciable quantities in all three states of matter (gaseous as water vapour, solid as ice and liquid). Since water is an integrated part of every living organisms, they are all dependent on its availability. Even human populations can only sustain themselves where there is a reliable freshwater supply (Moss, 2010). The growth of human populations worldwide is directly related with the exploitation of this natural resource (Herschy, 2012b); and, although more than two-thirds of Earth surface is covered by water, surface and useful water is relatively scarce. From the world's total water supply, over 96.5% is saline and, therefore, inappropriate form human consumption. Freshwater stocks represent only 2.5%, from which 68.7% is frozen in glaciers and permanent snows. The most reliable source of water for human consumption are the lakes and rivers, which represent only 0.26% of the freshwater resources (Gleick, 1998). In addition to the scarcity of this resource, freshwater in unevenly distributed over the globe and, for some areas, even through the year seasons (altering between drier and humid periods). This makes the division management of freshwater resources very complex politically and environmentally (Moss, 2010).

In addition to water scarcity, freshwater ecosystems are intensively explored and constantly exposed to many anthropogenic impacts (e.g. metals, pesticides, organic matter). The overexploitations of rivers leads to an increase of chemical, biological and geomorphological degradation. Channelization of the stream, sewage and industrial discharge, introduction of non-native species, drained water from irrigation and complete dryness are some of the most severe impacts caused by humans in lotic ecosystems. Damming is a particularly important human impact on rivers (Molles and Cahill, 1999). The growing requirement for water and energy supply derived from the development of human communities led to the increase of rivers damming (Moss, 2010). In terms of social and cultural needs, the constructions of dams was essential to produce energy, so this infrastructures were equipped with hydroelectric turbines allowed the storage of great quantities of water and also the production of hydroelectric power. Dams are also used to regulate the flow regime of the river, preventing floods and to ensure water supply during drought periods (Herschy, 2012a). However, such a great modification on the river natural course entails major consequences to aquatic biota. The obstruction of the river may compromise the integrity of the entire ecosystem, altering the water quality, food webs, seasonal variations of river flow and sediment transportation (McCartney et al., 2000).

The alteration and degradation of worldwide aquatic ecosystems by abusive human exploitation demanded an urgent creation of tools to analyse and monitor the present state of ecosystems and, also, predict future alterations. In response to this, documents such as European Water Framework Directive (WFD) where conceived. WFD is the most important directive in Europe concerning freshwater resources quality management and protection (Martinez-Haro et al., 2015b). According to this directive, all European water bodies must achieve a “good state” by 2015 (Navarro et al., 2009a), including rivers, lakes, transitional waters, coastal waters and heavily modified water bodies (which includes artificial water bodies such as reservoirs) (Martinez-Haro et al., 2015b).

Reservoirs

Reservoirs are artificial lentic waterbodies, formed in consequence of dams construction on the river bed (INAG, 2009b). These artificial ecosystems are similar to natural lakes in various aspects: the water storage and the low velocity of the streams, but they differ in aspects of geomorphology, annual and inter-annual storage variability, management options and catchment area (INAG, 2009b). Reservoirs have much larger level fluctuations than a natural lake and, also, dams usually have a bottom outlet that releases sediments and water from the depth of the reservoir, a phenomenon that is very rare in a natural ecosystem like lakes (McCartney et al., 2000).

Reservoirs can be divided, longitudinally, on three different zones (Figure I1): riverine, transition and lacustrine; according to variations in dynamics, physical, chemical and biological characteristics (Wetzel, 2001). Riverine zone is the most similar to the original system, with a narrow and shallow geomorphology typical from rivers. Although the velocity of the river flow starts to decrease in this zone, it is still strong enough to carry the smaller sediments and organic particles carried by the stream. This usually makes the water turbid and limits the primary production (Thornton et al., 1990; Wetzel, 2001). In the transition zone, velocity of the stream decreases, allowing significant sedimentation. This situation will increase light penetration in water and, consequently, primary production. Lacustrine zone is similar to a lake system in its characteristics. The sedimentation is low, with a reduction of light penetration, allowing higher rates of primary production (Thornton et al., 1990).

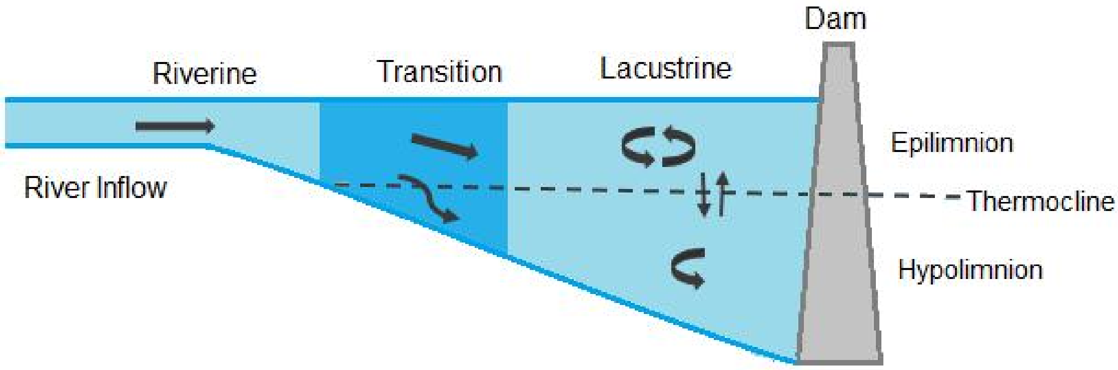


Fig. I1 – Longitudinal and vertical zonation in reservoirs (adapted from Thornton et al., 1990)

In deeper reservoirs may also occur vertical stratification (Figure I1), due to the variation of the temperature and light intensity from the surface to the bottom. The variation of temperature can form a thermocline (a sudden variation of the temperature) a few depth and separate the water column in layers that differ not only in temperature but also in physical, chemical and biological characteristics. The layer closer to the surface, above the thermocline, designated epilimnion, has higher temperature, dissolved oxygen concentration and the highest light intensity. In the epilimnion the water is well mixed by the warming and cooling of the surface and by the wind. The lower layer, underneath the thermocline, is the hypolimnion, has lower temperature, oxygen concentration and almost no light penetration. In this layer, there is low mixing of the water, only caused by the cooling of the water and due to discharges from the dam. Occasionally, some mixing of the waters from epilimnion and hypolimnion occurs, due to variations of the temperature (Farley, 2012).

In the past 4000 years a substantial number of dams have been constructed, to create artificial reservoirs, which were used to store water and regulate the variations in the stream. However, in the last two centuries this activity have increased exponentially, and their used has been amplified for other proposes (Wetzel, 1993). During the past 40 years, International Commission on Large Dams (ICOLD) registered a construction of more than 39000 large dams in the world (Herschy, 2012a). Dams are now constructed with many purposes, being the more usual for irrigation and water supply, in addition to the production of hydroelectric power (especially in Europe), flood control, recreation and fish farming (Herschy, 2012b). Portugal has 236 active dams, most of them explored by the group Energias de Portugal (EDP) for hydroelectric power production (Figure I2).

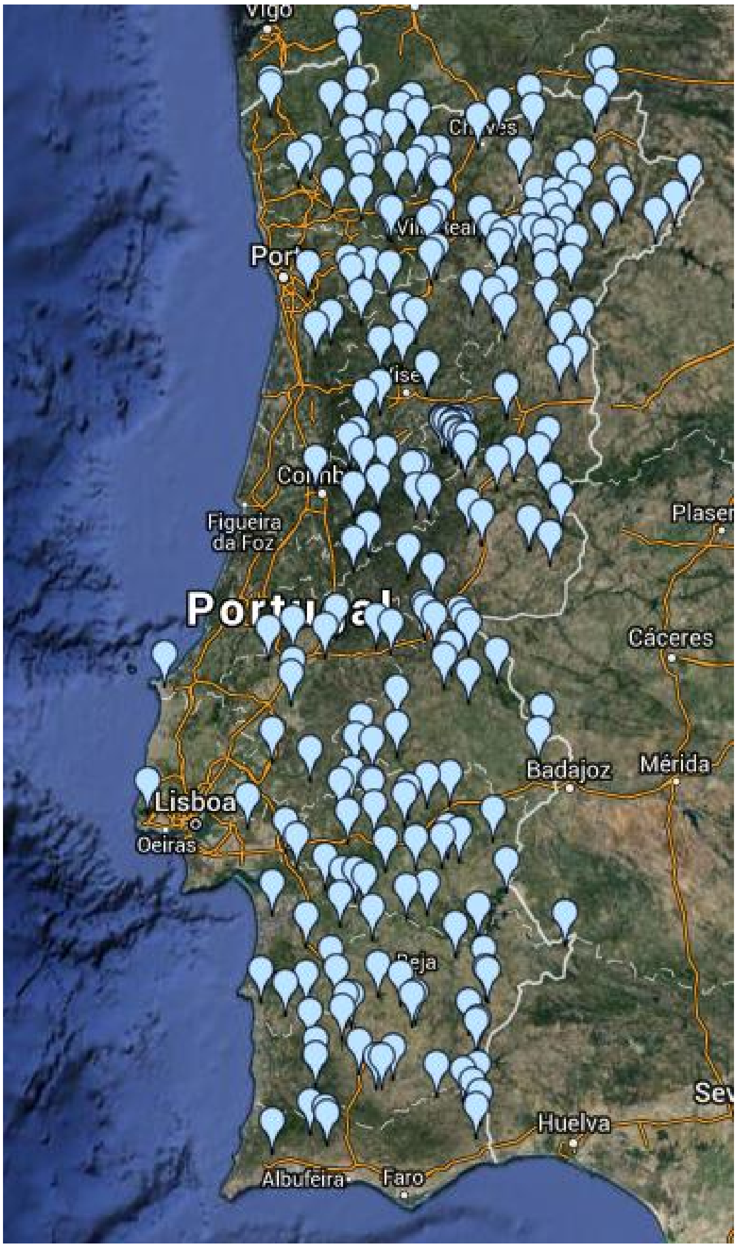


Fig. I2 – Location of all the active dams in Portugal (Source: Google maps)

Despite its importance in supporting the needs of human populations, the construction of dams entails major consequences to the ecosystem, both upstream and downstream of the dam. The most relevant is that the dam constitutes an interruption of the natural flow of the river stream and the fragmentation of the river, preventing migrations of species along the river course, and isolating the populations living up and downstream (McCully, 2001).

The accumulation of water upstream of the dam floods the terrestrial ecosystems, permanently destroying them. Besides the alteration in the landscape and its ecosystems, the flooding of the margins also compromises the quality of the water in the period after the closure of the dam, in result of the decomposition of the submerged marginal vegetation, originating dissolved oxygen depletion. The water stored in the reservoir is exposed to physical, chemical and biological changes, all which affecting the water quality. The concentration of nutrients such as phosphorus and nitrogen can increase, released from the flooded lands and released from the vegetation and soils. The enlargement of the surface area caused by the accumulation of water in the reservoir may also affect the quality of the water, particularly in terms of the concentration of nutrients, since more water can be lost due to evaporation (McCartney et al., 2000).

Concerning downstream effects, the stream flow variations through the year are compromised, caused by the control of the discharges from the dam. The flood peaks are reduced and, consequently, the inundation of the floodplain is controlled. The alteration in the flooding of the river banks and the disturbance of the natural hydrological system of the stream may have consequences on the riparian vegetation, and in the groundwater recharge. This regime flow regulation may have impacts in the ecosystems downstream of the dam. The water stored in the reservoirs suffers alterations in its quality and, when discharged by the dam, may affect the downstream ecosystems. It may differ in its composition and temperature from the natural seasonal pattern of the river. Temperature has been stated as an important parameter to evaluate the impact of a reservoir on the ecosystem, since it deeply affects physical, chemical processes and the biological communities of the river (McCartney et al., 2000).

The impact of a dam on the aquatic ecosystem is significantly dependent on human activities within the catchment area, such as industry, agriculture and animal farming. These activities can increase the loading of chemicals, nutrients, in particular phosphorus and nitrogen, with a consequence of degradation of the water quality, affecting the aquatic communities stablished in the reservoir, and may even cause alterations in downstream ecosystems (McCartney et al., 2000). Phosphorus and nitrogen are nutrients that are intensively used in agriculture, present in fertilizers and manures, that are heavily applied on farming soils and considerable quantities are latter drain to underground and surface waters. The excessive leaching of these nutrients to reservoirs can lead to eutrophication process (Smith et al., 1999a) (Figure I3). Eutrophication is a consequence of a significant increase concentration of nutrients (in particular phosphorus and nitrogen) in water bodies, that causes an abnormal growth of primary producers (phytoplankton and aquatic plants), which can compromise the quality of the water and the balance of the ecosystem (Farley, 2012). The exponential growth of phytoplankton increases the photosynthesis rate in the epilimnion area and, consequently a significant decrease of oxygen concentration and pH values. It also increases the water turbidity, which reduces light penetration in the lower layers of water, leading to the death of the aquatic plants and microalgae in the bottom of the reservoir. On the other hand, the decomposition of the organic matter causes an oxygen depletion in the hypolimnion layer which conduce at water degradation (Moss, 2010).

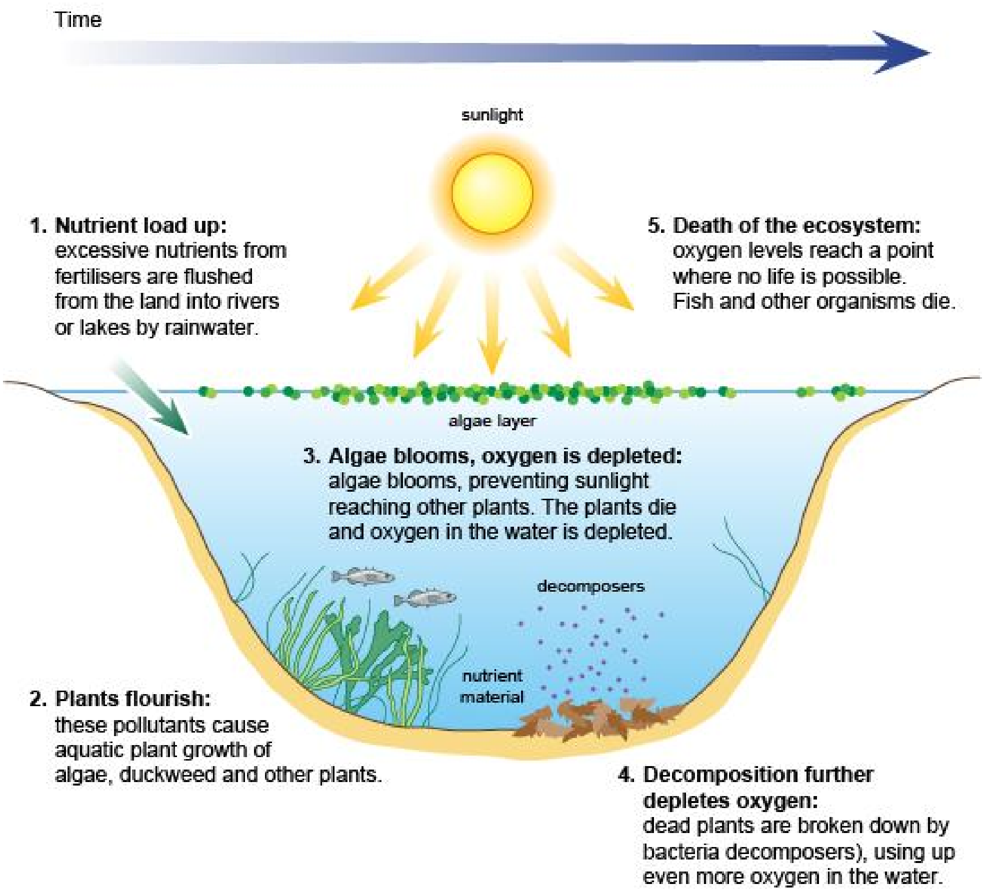


Fig. I3 – Representative scheme of the process of eutrophication in lakes (source: http://www.bbc.co.uk/schools/gcsebitesize/science/edexcel/problems_in_environment/pollutionrev4.shtml)

An usual methodology for classification of water bodies is the trophic state, using the Trophic State Index (TSI) (Carlson, 1977). This index classifies the water bodies in four categories, according to their nutrient enrichment and the effects of the water quality:

- *Oligotrophic*: clear waters, high concentration of dissolved oxygen in hypolimnion, high transparency and light penetration, low nutrient supplies, low primary productivity.
- *Mesotrophic*: moderately clear waters, intermediate nutrient supplies, medium primary productivity.
- *Eutrophic*: heavy nutrient supply, high primary productivity, low transparency and light penetration, low concentration of dissolved oxygen in hypolimnion.
- *Hypertrophic*: excessively high nutrient supply, waters extremely eutrophic, very low transparency.

According to Carlson (1977), TSI can be obtained using several common parameters to access water quality, such as water transparency, using data collected with Secchi disk, content of chlorophyll *a* and total phosphorus concentration (Table I1). These parameters are used in their correspondent equations to calculate TSI were:

- Total phosphorus: TSI-P = 14.42 * Ln [TP] + 4.15 (in µg/L)
- Concentration of chlorophyll *a*: TSI-Chl= 30.6 + 9.81 Ln [Chl-*a*] (in µg/L)
- Transparency: TSI-T = 60 - 14.41 * Ln [Secchi] (in meters)

Average TSI is calculated by the average of all the three values:

Average TSI = (TSI-P + TSI-Chl + TSI-T)/3

Table I1: Trophic State Index classification for lakes (adapted from Smith et al., 1999)

Trophic State	**Transparency (Secchi disk - m)**	**Concentration of Chl *a* (mg m^{-3})**	**Total Phosphorus (mg m^{-3})**
Oligotrophic	>4	<3.5	<10
Mesotrophic	2-4	3.5-9	10-30
Eutrophic	1-2	9-25	30-100
Hypertrophic	<1	>25	>100

TSI can also be determine through a graphical approach, using a value scale to classify the water body (Figure I4).

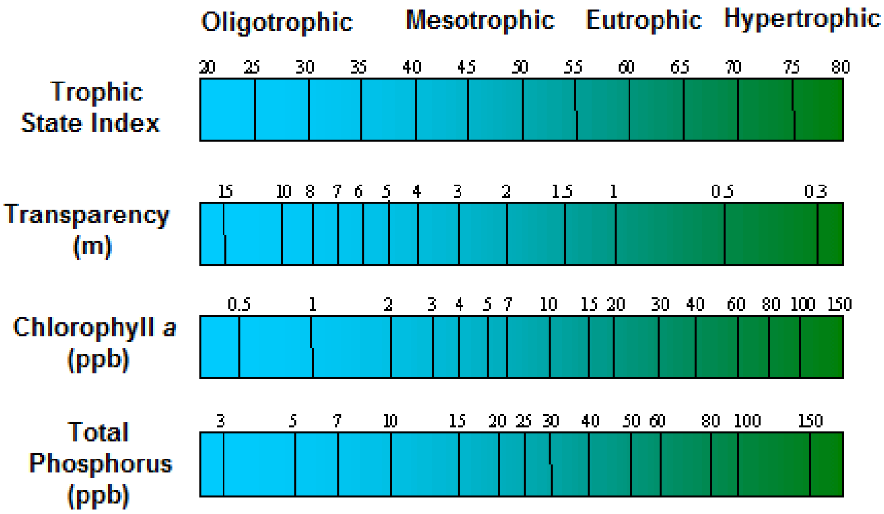


Fig. I4 – Graphical presentation of TSI (source: http://www.lakeaccess.org/lakedata/datainfotsi.html)

Water Framework Directive

Under the matter of legislation for water resources management, the Water Framework Directive (WFD) constitutes one of the most important documents implemented by European Union. This directive was created in 2000 by European Commission and states that all signing Member States must protect, restore and preserve all the water resources, aiming to achieve a "Good Status" for all the inland surface, transactional, coastal and ground waters by 2015 (Martinez-Haro et al., 2015b). This directive was transposed to the national legislation by the DL 58/2005 in December 29th (Lei da Água) and the DL 77/2006 in March 30th (INAG, 2009a).

WDF proposes the use of stipulated criteria of physical, chemical, biological and hydromorphological parameters to assess the ecological status of a water body through provided guidelines for each parameter. Using reference values stated by the directive, the water bodies are classified under five classes: high, good, moderate, poor and bad quality (Navarro et al., 2009a). The WFD represents an improvement of previously existing tools for monitoring and conservation of aquatic ecosystems. Since it demands a multidisciplinary approach to assess the quality of the water body, it requires a deeper understanding on the structure and functionality of the ecosystem. The "Status" of an aquatic system requires the combined evaluation of both "Ecological

Status" and "Chemical Status", and the lowest of the two determines the overall "Status" (Martinez-Haro et al., 2015b). According to WFD, Ecological Status is defined as the structural and functional quality of the ecosystems and is rated according to the deviation from the reference conditions, where the ecosystem was not exposed to anthropogenic disturbance. However, reservoirs are artificial water bodies, originated by physic anthropogenic interference in the river. Therefore, WFD classifies them as "Heavily Modified Water bodies", which defines water bodies that, due to physic alterations resulting from human activities, acquired different characteristics compared to the original system. For heavily modified water bodies, instead of "Ecological Status", WDF proposes the evaluation of the "Ecological Potential". This nomenclature means the deviation from the "Maximum Ecological Potential" that the ecosystem can achieve after being implemented all the possible mitigation measures without adverse consequences on the system and on the environment (Borja and Elliott, 2007). Since there are no reference values for systems classified as heavily modified, the directive proposes the use of maximum thresholds values for the most similar system to evaluate the ecological potential, which for the case of reservoirs means natural lakes.

Reservoirs are also classified under categories, according to their geographical and river course location (Figure I5). There are three different types of reservoirs in continental Portugal:

- Northern reservoirs: located in mountainous areas with granitic subtract, in regions with high annual rainfall and average annual temperature inferior to 15ºC. They are mostly used for production of hydroelectrically power and they have a residence time inferior to 7 months.
- Southern reservoirs: located in lowland areas with subtract of shale and sedimentary rocks, in regions with low annual rainfall and average annual temperature superior to 15ºC. These reservoirs are mostly used for irrigation and water supply, with high residence time (superior to 7 months).
- Main course reservoirs: located in the main courses of Douro, Tejo and Guadiana rivers. With very low residence times, inferior to 10 days and a high drainage area.

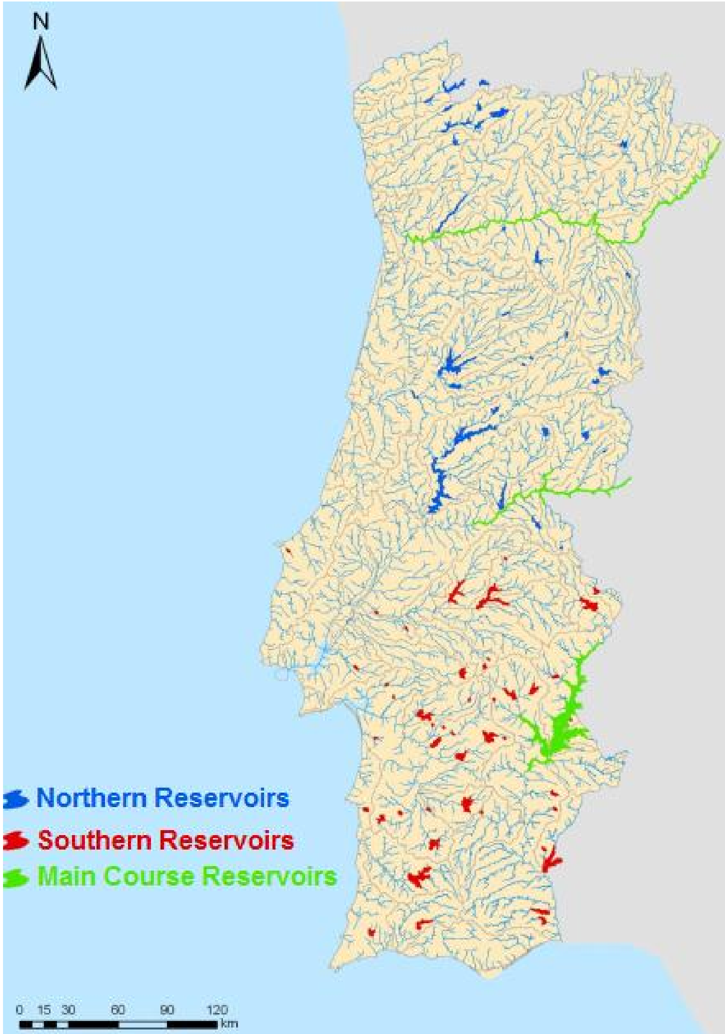


Fig. I5 – Location of the northern, southern and main course reservoirs in Portugal (source: http://www.apambiente.pt/dqa/assets/tipologia-de-massas-de-%C3%A1gua-fortemente-modificadas---albufeiras.pdf)

Due to the specificity of their characteristics, the methodologies and parameters stated for ecological potential cannot be applied for main course reservoirs (INAG, 2009a). Thus WFD, proposes reservoirs evaluation to considering the ecological potential based on the quality of biological, physical and chemical and hydromorphological elements (Borja and Elliott, 2007).

Physical and Chemical Elements

The WFD proposes a series of physical and chemical parameters and the correspondent methodologies, which should be used to monitor the water quality in the

"Heavily Modified Water bodies". In the case of reservoirs, the elements stated for evaluation of ecological status in lakes should be used to assess the ecological potential in reservoirs (Table I2).

Table I2: General physical and chemical parameters to monitor in heavily modified water bodies – reservoirs (adapted from INAG, 2009a)

General Physical and Chemical Elements	**Parameters**	**Unities**
Transparency conditions	Secchi depth	m
	Total suspended solids	mg/L
	Color	Pt/Co scale
	Turbidity	NTU
Thermal conditions	Temperature profile	ºC
Oxygenation conditions	Dissolved oxygen profile	mg O_2/L
	Oxygen saturation rate profile	% O_2 saturation
	Biochemical oxygen demand	mg O_2/L
	Chemical Oxygen Demand	mg O_2/L
Salinity	Electrical conductivity at 20ºC (average)	µS/cm
Acidification status	pH	Sorensen scale
	Alkalinity	mg HCO_3/L
	Hardness	mg $CaCO_3$/L
Conditions on nutrients	Nitrates	mg NO_3/L
	Nitrites	mg NO_2/L
	Ammonium	mg NH_4/L
	Total nitrogen	mg N/L
	Orthophosphate	mg PO_4/L
	Total phosphorus	mg P/L

Due to the lack of historical data, it was impossible to define the boundaries for all the three classes (excellent, good and moderate) for the evaluation of physical and chemical elements. So far it has only been possible to stablish the upper and lower boundaries for the "good ecological potential" for reservoirs, presented here in Table I3.

Table I3: Maximum thresholds for physical and chemical parameters for Good Ecological Potential in heavily modified water bodies – reservoirs (adapted from INAG, 2009a)

Parameters	**Limit to Good Potential**	
	Northern Reservoirs	**Southern Reservoirs**
Dissolved oxygen[(a)]	≥5 mg O_2/L	≥5 mg O_2/L
Oxygen saturation rate[(a)]	Between 60% and 120%	Between 60% and 140%
pH[(a)]	Between 6 and 9	Between 6 and 9
Nitrates[(b)]	≤25 mg NO_3/L	≤25 mg NO_3/L
Total Phosphorus[(b)]	≤0.05 mg P/L	≤0.07 mg P/L

(a) 80% of the samples if the sampling events are monthly or superior
(b) Annual average

Hydromorphological Elements

Within the WFD, hydromorphological elements allow to determine the reference conditions of the water bodies. These parameters define the quality targets to evaluate the ecological status, to pre-determine the type of waterbody and to assess them in terms of current status achievement. Hydromorphological elements are of great importance in the ecosystem, since they represent the abiotic support for all the biological component (Weiß et al., 2008). The two hydromorphological elements for reservoirs evaluation in this directive are the hydrological regime and the morphological conditions (Table I4). The hydrological regime represents all the variations in the water flow. The morphological conditions concern all the characteristics of the water body, such as the depth, the type and the quantity of the substrate and the banks structure.

Table I4: Hydromorphological elements and their respective component and indicator to assess the Ecological Potential in heavily modified water bodies – reservoirs (adapted from INAG, 2009a)

Hydromorphological Elements	**Component**	**Indicator**
Hydrological regime	River flow and outflow conditions	Affluences, captured flow, powered, discharged, water level
	Residence time	Residence time
	Connection to groundwaters	-
Morphological conditions	Depth variation	-
	Quantity, structure and substrate bed	
	Structure of banks	

Biological Elements

The WFD establishes the use of biological elements and respective components to assess ecological status and ecological potential. Concerning the biological parameters, WFD demands an intercalibration exercise, in order to homogenise the reference values for "good state" and respective boundaries for all the States Member and to allow comparisons between different locations with the same types of water bodies. To perform this intercalibration exercise, all States Member were organized under groups according to the characteristics of their water masses, called Geographical Intercalibration Groups (GIG). Portugal integrated the Mediterranean GIG. For lakes, the original proposal included four different quality elements: benthic invertebrates, fish fauna, phytoplankton and other aquatic flora. Although the directive proposes more elements, the intercalibration exercise has only stated thresholds values for phytoplankton community. This element should be assessed in terms of composition, abundance and biomass (Table I5).

Although, for reservoirs, WFD proposes only the evaluation of phytoplankton (main primary producer in aquatic ecosystems) to assess water quality. Consumers such as fishes and zooplankton are not included in WFD for water classification. Nevertheless, zooplankton is a primary consumer of high importance in every aquatic ecosystem, highly susceptible to biological, physical and chemical alterations in the ecosystems (An, 2012; Azevêdo, 2015). According to this point a view, zooplankton can be used as a biological quality element for the evaluation of the Ecological Status.

Table I5: Indicators to evaluate the biological elements in heavily modified water bodies – reservoirs (adapted from INAG, 2009a)

Biological Element	**Component**	**Indicator**	**Reservoir Type**
Phytoplankton*	Composition and abundance	Algae group index	Northern
		% of cianobacteria biovolume	Northern
	Biomass	Concentration of Chlorophyll *a* (mg/m^3)	Northern and Southern
		Total biovolume (mm^3/L)	Northern

*For Northern reservoirs, only average summer values are used. For Southern reservoirs, annual average values are used in the evaluation.

Zooplankton

Zooplankton represents the group of small, heterotrophic organisms that live drifting in the water bodies. Zooplankton plays a key role in the food webs of lentic aquatic ecosystems. As primary consumers, these communities have an important part in the flow of matter and energy between the phytoplanktonic producers and planktivorous fishes (Abrantes et al., 2006; Jensen et al., 2013). They are also responsible for the water body capacity of self-purification, since they feed on suspended particles, and nutrient sequestration (An et al., 2012; Li et al., 2014). Zooplankton biota is mostly constituted by three groups of organisms: the class Rotifera and two suborder of Crustacea, Copepoda and Cladocera (Wetzel, 1993).

Rotifera is the greatest class of zooplankton, with a great number of described species, particularly for freshwater environments. The organisms belonging to Rotifera class are usually very small, with a ciliated corona on the front head, which allows the organism to dislocate and to conduct the food particles to the mouth (Wetzel, 1993).

The organisms that belong to subphylum Crustacea are almost all aquatic. Their body is divided under three segments, with a tendency to merge the abdomen and the thoracic areas, especially in suborder Cladocera. As all the Arthropoda, crustaceans have articulated appendixes, either they are exposed or covered by a shell. Planktonic freshwater crustacean communities are majorly dominated by Cladocera and Copepoda (Wetzel, 1993), and they represent the largest component of zooplankton biomass (Semenova and Aleksandrov, 2009).

Cladocerans are small animals, usually with only a few millimetres in length, with the body covered by a bivalve shell (Figure I6). They use their second pair of antennae to dislocate in water. These animals are mostly herbivorous, filtering the water using thoracic appendixes to catch small suspended particles and phytoplankton, but some genus are carnivores, feeding smaller zooplankton (Moss, 2010). These animals have parthenogenetic reproduction, with populations being constituted only by females most of the year, which produce parthonegic eggs in the brood chamber. The eggs mature inside the chamber and the offspring are released during the moult. Cladocerans species do not have larval forms, except for the genus *Leptodora*. In case of shifts in environmental conditions, such as alterations in temperature, light period or a decrease in the availability or quality of the food, Cladocerans may reproduce sexually. The reproduction and lifetime of cladocerans may be affected by temperature and other parameters (e.g. pH or contamination). The increase of temperature induces the organisms to increase their moult rates and, consequently, their production of offspring (Wetzel, 1993).

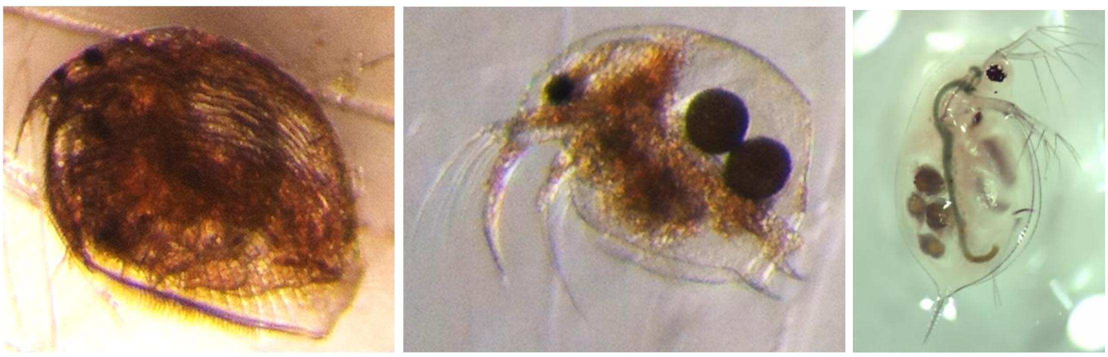


Fig. I6 – Cladocerans organisms. Left: *Alonella sp.* Middle: *Bosmina sp.* with two eggs in the brood chamber; Right: *Daphnia longispina* with offsprings in the brood chamber.

Organisms that belong to Copepods suborder can be separated under three different groups: Harpaticoida, Cyclopoida, and Calanoida (Figure I7). They are usually the largest animals on zooplanktonic communities. Some of them feed small particles (most of the calanoids) and others are predators (which is the case for cyclopoids), feeding smaller zooplankton, colonies and aggregates of phytoplankton and detritus. Contrary to cladocerans, copepods have the capability of selecting the food particles among those which get near the animal's mouth. They are also the capacity to feed larger particles then the other elements of the zooplankton (Moss, 2010). Copepods reproduce sexually, where the male transfers its spermatophores during copulation. The female carries the fertilized eggs in 1 (in calanoids and harpaticoids) or 2 (in cyclopoids) egg sacs. The size of the brood produced varies during the year seasons. The larger broods are produced in spring and autumn, due to an increase of the primary productivity in the ecosystem. The egg hatches a larval form named nauplii, which develops into adult state through successive moults (Wetzel, 1993).

Fig. I7 – Copepoda organisms. Left: Cyclopoida; Middle: Calanoida (source: http://www.micromagus.net/animalcules/copepoda/diaptomus04.jpg); Right: Harpaticoida (source: http://microlife.parvarium.com/FC0809/Arthropod1479.jpg)

The zooplankton community's distribution and abundance are highly dependent on various environmental factors. Firstly, it can be affected by biological aspects. It has been proven that zooplankton community is strongly influenced by both bottom-up and top-down processes, being strongly dependent on the nutrient availability and abundance of phytoplankton, and also on predation from fishes and macroinvertebrates (Abrantes et al., 2006). The size of the organisms, as well as the species composition is a reflex of the biological pressures on the zooplanktonic community (An et al., 2012). In addition to this, the structure and biodiversity of the zooplankton communities is also deeply dependent by abiotic factors, such as temperature, pH, organic carbon (Jensen et al., 2013), fluctuations in the water level (Geraldes and Boavida, 2007) and turbidity (Li et al., 2014). Thus, the structure of the zooplankton community's is a reflection of the functional proprieties of the aquatic ecosystems (Azevedo et al., 2015; Castro et al., 2005; Jensen et al., 2013).

In the light of the above stated, some authors have discussed the possibility of the use of zooplankton as a biological quality element for the evaluation of the Ecological Status of the water bodies proposed in the WFD (Caroni and Irvine, 2010; Jensen et al., 2013; Jeppesen et al., 2011b) and they suggest its inclusion in the Biological Quality Elements proposed by the WFD, namely for "Heavily Modified Water bodies".

Main objectives

In light of the information presented in here about WFD and the scarce information on the role of zooplankton in defining water quality, the present project intends to:

- Assess and compare the water quality in four reservoirs of the Cávado's hydrographic basin, according to WFD approach and to the community composition, diversity and abundance of zooplankton communities.

To achieve this main goal, some more specific objectives were stablished to address the problem:

- To analyse the seasonal variation of the physical and chemical elements of the water quality in each reservoir.
- To analyse the zooplankton communities in each reservoir, to evaluate their potential as a biological element to assess the ecological potential in reservoirs.

Material and Methods

Study Area

Portuguese territory is divided under 8 hydrographic regions (Fig. MM1) and all hydrographic regions have their own management plan, Plano de Gestão da Região Hidrográfica - PGRH.

- RH1 – Hydrographic Region of Rivers Minho and Lima
- RH2 – Hydrographic Region of Rivers Cávado, Ave and Leça
- RH3 – Hydrographic Region of Douro River
- RH4 – Hydrographic Region of Rivers Vouga, Mondego and Lis
- RH5 – Hydrographic Region of Tejo River
- RH6 – Hydrographic Region of Rivers Sado and Mira
- RH7 – Hydrographic Region of Guadiana River
- RH8 – Hydrographic Region of Algarve Streams

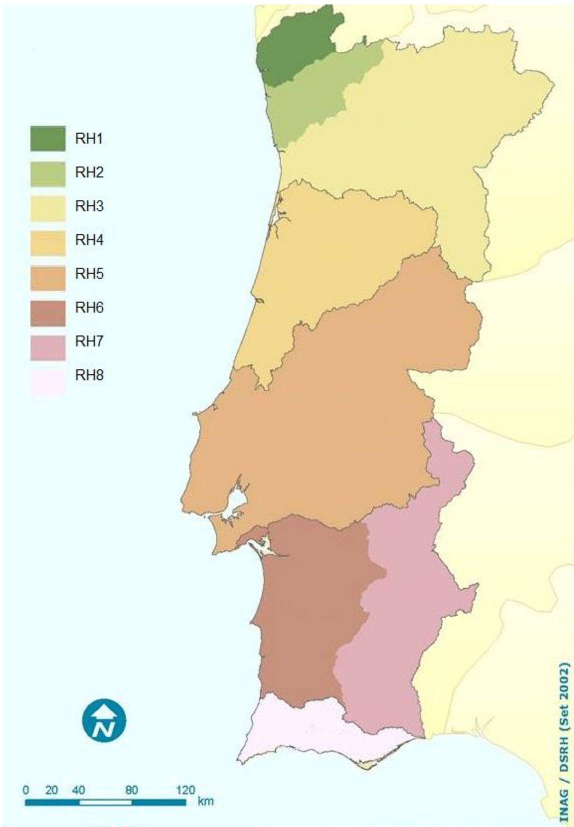


Fig. MM1 – Hydrographic regions in Portugal Continental (Source: http://snirh.apambiente.pt/index.php?idMain=4&idItem=2&idISubtem=3)

The artificial reservoirs chosen for this study were located in the northern area of Portugal, in the oriental domain of the Hydrographic Regions of Rivers Cávado, Ave and Leça - RH2 (Figure MM2A).

Hydrographic Regions of Rivers Cávado, Ave and Leça (RH2) is located in the Northeast of Portugal, being limited at North by Hydrographic Region of Rivers Minho and Lima (RH1) and Spain, by Hydrographic Region of Douro River (RH3) at South and East, and by Altantic Ocean at West coast. RH2 covers an area of approximately 3,400 km^2, and involves 4 districts: Braga, Porto, Viana do Castelo and Vila Real. Braga has the greatest area in the hydrographic region and Porto has the higher population density. This hydrographic region is divided in 4 different hydrographic sub-basins: Cávado, Ave, Leça and coastal between Neiva and Douro (INAG, 2012). Cávado's hydrographic basin is the greatest sub-basin of RH2. This region has an area of 1,593 km^2, covering the municipalities of Amares, Barcelos, Boticas, Braga, Cabeceiras de Basto, Esposende, Montalegre, Ponte da Barca, Ponte de Lima, Póvoa do Lanhoso, Póvoa de Varzim, Terras de Bouro, Vieira do Minho and Vila Verde. Although its great geographic area, it has the lowest population density of all the sub-basins of that Hydrographic region. The main water course is the Cávado River, with a length of approximately 129 km. It springs in Serra do Larouco, in Spain, and meets the Atlantic Ocean in Esposende. Cávado River main tributaries are Homem River and Rabagão River (INAG, 2012). This region is typically a mountainous area, characterized by its steep slopes and deep valleys, with a planned bottom. Granitic bedrock is predominant in this area. This eastern limit of Cávado's river hydrographic basin shows a relatively high rainfall average (approximately 2200 mm/year) and an annual average temperature of 9.9ºC.

Four reservoirs were chosen: Venda Nova, Alto Rabagão, Alto Cávado and Paradela, belong to the Cávado's river hydrographic basin (Figure MM2B). All the reservoirs studied are located in a rural area, with a very low population density, and close to the protected natural area of National Park of Peneda-Gerês, being two of them, Alto Cávado and Paradela, located in its eastern limit (INAG, 2012).

Fig.MM2 – A) Location of the study area in the Portuguese territory; B) Detail of the location of the studied reservoirs. (source: Google Maps)

All the dams of the reservoirs of this study are explored and maintained by Energias de Portugal - EDP for production of hydroelectric power. Nowadays, the selected reservoirs has not yet been approved for a Plano de Ordenamento de Albufeiras – POA. Venda Nova, Alto Rabagão and Paradela are considered hydroelectric exploitations of great dimensions.

Venda Nova reservoir (Figure MM3) is inserted in a section of the Rabagão River, located in the municipality of Vieira do Minho, in the Braga district. According to SNIRH database, the dam was inaugurated in 1951, measures 97 m height and has a total capacity of 94,500 dam^3. The water of this reservoir is mostly used for agriculture and for domestic and urban supply. In this reservoir two sampling sites where initially selected, but due to great variations in the water level (Figure MM4) it was impossible to reach the water in the second site for most of the sampling period, so it has been excluded from the study. Thus, only one sampling site was ultimately chosen for Venda Nova, located close to the dam wall (site 1: 41º40'56.021"N; 07º58'56.056"W) (Figure MM3).

Fig. MM3 – Left: Sampling sites 1 and 2 (excluded) located in Venda Nova reservoir (source: Google Maps); Right: Sampling site 1 (photography by Rafaela Almeida)

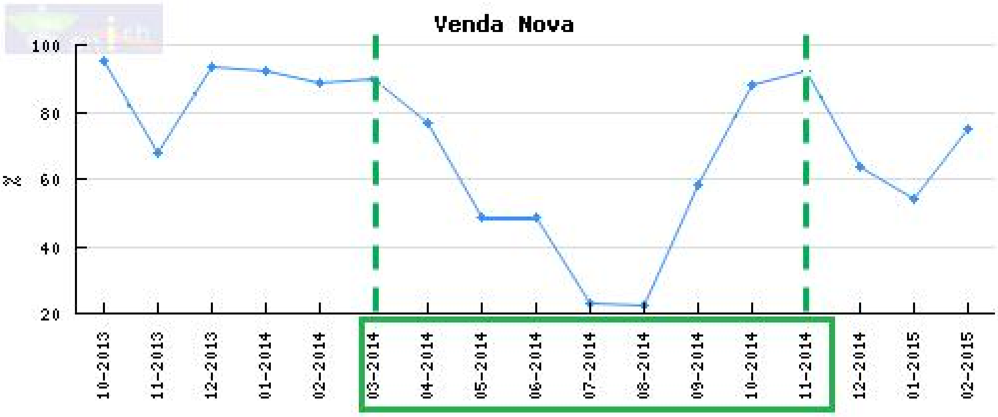


Fig. MM4 – Variation in the water level in Venda Nova reservoir (adapted from SNIRH). In green is evidenced the sampling period.

The reservoir of Alto Rabagão (Figure MM5) is the first in the course of Rabagão River. Is located in the municipality of Montalegre, in the district of Vila Real. It is located upstream from the reservoir of Venda Nova, reservoir which receive water discharges of Alto Rabagão. The dam started working in 1964, and this reservoir is majorly explored for energy production purposes, although it is also used by a trout farming and for recreation. Such as Venda Nova, the water is mostly used for agriculture and for domestic and urban supply. Alto Rabagão is the largest reservoir among the four selected for this study, with a total capacity of 568,690 dam^3 and a height of 94 m. Considering its great dimensions, three sampling sites were primarily chosen for this reservoir. However, the water column changes made it impossible to collect data and samples in one of the sampling sites for a few months, so to avoid gaps in the results analysis, this third site was removed from the study. The two remaining sampling sites were located in distinct areas of the reservoir, the first one near of the water input channel from Alto Cávado reservoir (site 4: 41º45'06.372"N;

07º51'0.547"W) (Figure MM6) and the other close to the trout farming (site 5: 41º45'10.808"N; 07º52'08.771"W) (Figure MM6).

Fig. MM5 - Sampling sites 4, 5 and 6 (excluded) located in Alto Rabagão reservoir (source: Google Maps)

Fig. MM6 – Left: Sampling site 4 (photography by Rafaela Almeida); Right: Sampling site 5 (photography by Rafaela Almeida)

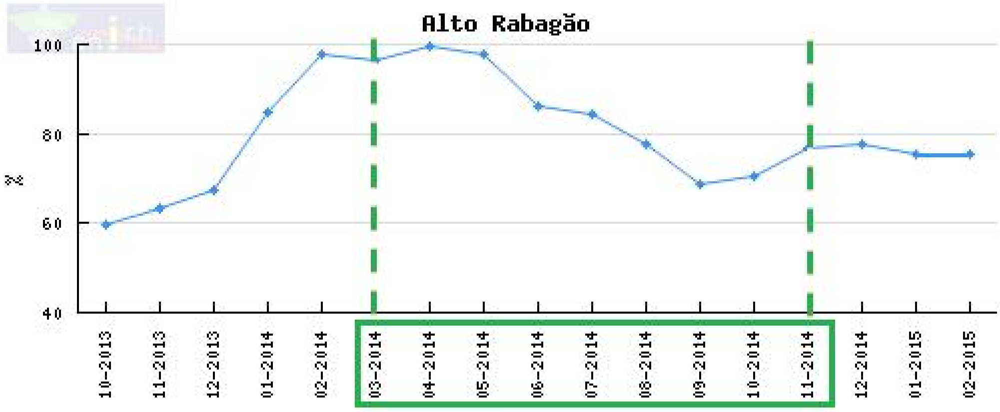


Fig. MM7 - Variation in the water level in Alto Rabagão reservoir (adapted from SNIRH). In green is evidenced the sampling period.

The reservoir of Alto Cávado (Figure MM8) is located in Cávado River, in the municipality of Montalegre, district of Vila Real. Although it is located upstream from the reservoir of Paradela, it does not discharge directly to it, but it does discharge to the reservoir of Alto Rabagão. The dam was inaugurated in 1964, measuring 26 m height and with a total capacity of 3,300 dam^3 of water. This reservoir is the only one among the four selected that has a concession for sport fishing, legalized by the National Forestry Authority. For this reservoir, one sampling site was select, close to the dam wall (site 8: 41º48'06.122"N; 07º52'32.956"W) (Figure MM8).

Fig.MM8 – Left: Sampling site 8 located in Alto Cávado reservoir (source: Google Maps); Right: Sampling site 8 (photography by Rafaela Almeida)

The last reservoir is Paradela (Figure MM9), which is located downstream from Alto Cávado in Cávado River. Is located in the municipality of Montalegre, in the district of Vila Real. The dam started working in 1956, has 112 m of height and the reservoir holds a total capacity of 164,390 dam^3. Once this reservoir is very isolated, and its water is only used for agricultural purposes. One sampling site was chosen in this reservoir (site 9: 41º46'22.521"N; 07º57'37.203"W) (Figure MM9).

Fig. MM9 – Left: Sampling site 9 located in Paradela reservoir (source: Google Maps); Right: Sampling site 9 (photography by Rafaela Almeida)

Sampling Procedures

In situ

Sampling period was carried out in 2014, between March and November. Monthly sampling events were performed. In every sampling event, a multi-parameter probe, WTW Multi 350i (Figure MM10A), was used to register *in situ* parameters: temperature, dissolved O_2 (mg/L and %), pH, conductivity (µS/cm) and total dissolved solids (mg/L). In each sampling site water samples were collect ate the surface in plastic bottles for further analysis of chemical and physical parameters in the laboratory (photosynthetic pigments and suspended solids, as well as nutrients, and biochemical oxygen demand). Zooplankton samples were collect using a net (150 µm) (Figure MM10B) and performing five horizontal hauls below the surface. Zooplankton samples were preserved in alcohol at 96% for later identification and count.

Fig.MM10 – A) WTW Multi 350i multi-parameter probe; B) Zooplankton net (photographies by Rafaela Almeida)

Laboratory procedures

The water collect during the sampling events was brought to the laboratory in thermal bags and in the dark to analyse physical and chemical parameters included in Water Framework Directive.

- *Total suspend solids (TSS)* and *turbidity* are parameters that evaluate the water transparency conditions. As described by APHA (1985), to determine total suspended solids, the sampled water was filtrated through a glass microfiber filter with a 1.2 µm porosity, 47 mm diameter (Whatman GF/C filter), using a vacuum pump (Figure MM11) until the filters were completely full. Clean filters were weighted to determine the average clean weight. The used filters were left in the heater (60ºC) until completely dry. After this period, the filters were weighted and compared to the average clean weight. Turbidity was tested using a spectrophotometer calibrated at 450 nm to measure non filtrated water sample, according to the procedure described by Brower J.E. et. al (1998).

Fig. MM11 – Filtration system (photography by Rafaela Almeida)

- *Chlorophyll* a is a parameter that measures the concentration of this photosynthetic pigment present in the water sample. As stated by the method described by Lorenzen (1967), the water sampled was filtrated using the same procedure as the one used for TSS, and the particles collected in filter were then ground in 90% acetone and stored in the dark at 4ºC for 24 h, to complete pigment extraction. Extracts were read at 665 nm and 750 nm in a spectrophotometer before and after acidification with HCl 0,1 M. Chlorophyll *a* concentrations, expressed in mg m^3, were calculated according to Lorenzen's monochromatic equations.

$$\text{Chl } a = \frac{26.7*(E665o - E665a)*v}{V*l}$$

E665o = ABS_{665} – ABS_{750}; E665a = ABS_{a665} – ABS_{a750}; v – acetone volume used in the extraction process (mL); V – volume of filtered water (L), l - optical cuvette path (cm).

- *CDOC* (Organic Carbon Dissolved) is a parameter that allows to evaluate the concentration of organic carbon dissolved, the major reservoir of carbon in natural waters, present in the water sample. According to the methodology stablished by Williamson et al. (1999), the filtrated water sample was read on a spectrophotometer at 320 nm, using quartz glass cuvettes and CDOC was calculated using the equation:

$$\text{CDOC} = \frac{2.30 * ABS320}{l}$$

ABS_{320} – Absorbance values for 320nm; l –optical cuvette path (0.01m).

- *BOD_5* (Biochemical Oxygen Demand) is the measure of the amount of oxygen required to oxidize the organic matter present in the water sample after 5 days of incubation of controlled conditions. To evaluate this parameter, the oxygen concentration (mg/L) in water samples was measured in the sampling day (Day_0) (Figure MM12). Then, ambar glass bottles with water samples were stored at 20ºC in the dark for 5 days. Allylthiourea was added to the water as a nitrification inhibitor. At the end of this period, oxygen concentration is measured (Day_5) and BOD_5 was calculate according to the equation:

$$BOD_5 = Day_0 - Day_5$$

Fig. MM12 – Measurement of oxygen concentration in water samples using a multi-parameter probe (photography by Rafaela Almeida)

- *Nitrates, nitrites*, *ammonia* and *total phosphorus* are parameters that evaluate the presence and/or concentrations of nutrients in the water. To evaluate nitrates and phosphorus, the water was primarily mineralized with $K_2S_2O_8$. Nitrates were measured using a *Hanna Instruments* model C200 spectrophotometer, with a procedure based on an adaptation of the cadmium reduction method. Total phosphorus was measured according to the methodology described by APHA (1985). Mineralized water samples react with ammonium molybdate and are reduced by tin chloride, acquiring a blue colour. This solution was read on the spectrophotometer at 690 nm and total phosphorus was quantified according to a standard calibration curve. Nitrites and ammonia were quantified in non-filtrated water samples, using a *Merck KGaA* Spectroquant colorimeter and correspondent test kits.

Zooplankton

Zooplankton samples were identified using a standard binocular magnifying glass (Fig. MM13). Cladoceran and Copedoda taxa were identified and counted using proper identification keys: Amoros (1984), Alonso (1996) and Harding and Smith (1974). All the samples were fully identified and counted with the same resolution for all taxa.

Fig. MM13 – Stand binocular magnifying glass (photography by Rafaela Almeida)

Statistical analysis

To analyse physical and chemical parameters, a Principal Components Analysis (PCA) was performed, using the software Canoco for Windows 4.5. PCA is a form of multivariate analysis, which allows to assess possible correlations between different variables in a matrix (Ter Braak and Van Tongeren, 1995).

The structure and composition of zooplankton communities were analysed through descriptive statistical methods, using Microsoft Office Excel. This allowed us to determine the relative abundance of the species identified for each sample. Since our sampling method did not allow obtaining an exact volume of sample, the zooplankton data was analysed under a semi-quantitative perspective.

To analyse possible correlations between zooplanktonic communities and environmental data, a Canonical Correspondence Analysis (CCA) was performed, also using the Canoco software. The CCA allows to correlate the biotic and environmental data from different matrices and to represent quantitatively explanatory variables in a graphic (Abrantes et al., 2006).

Results

Physical and Chemical parameters

Physical and chemical data obtained for all the sampling sites and sampling period (see *Annex I*) was compiled on a single matrix, which was used to perform a Principal Components Analysis - PCA (Figure R1). In addition to the physical and chemical elements stated by the WFD to be assessed in the reservoirs, some additional parameters were analysed in this study. The physical and chemical data assessed were able to explain a total variance of 33.8% of the sampling sites and sampling period. This low percentage can be explained by the great quantities of environmental variables that couldn't be assessed, such as specific pollutants and changes in the water level.

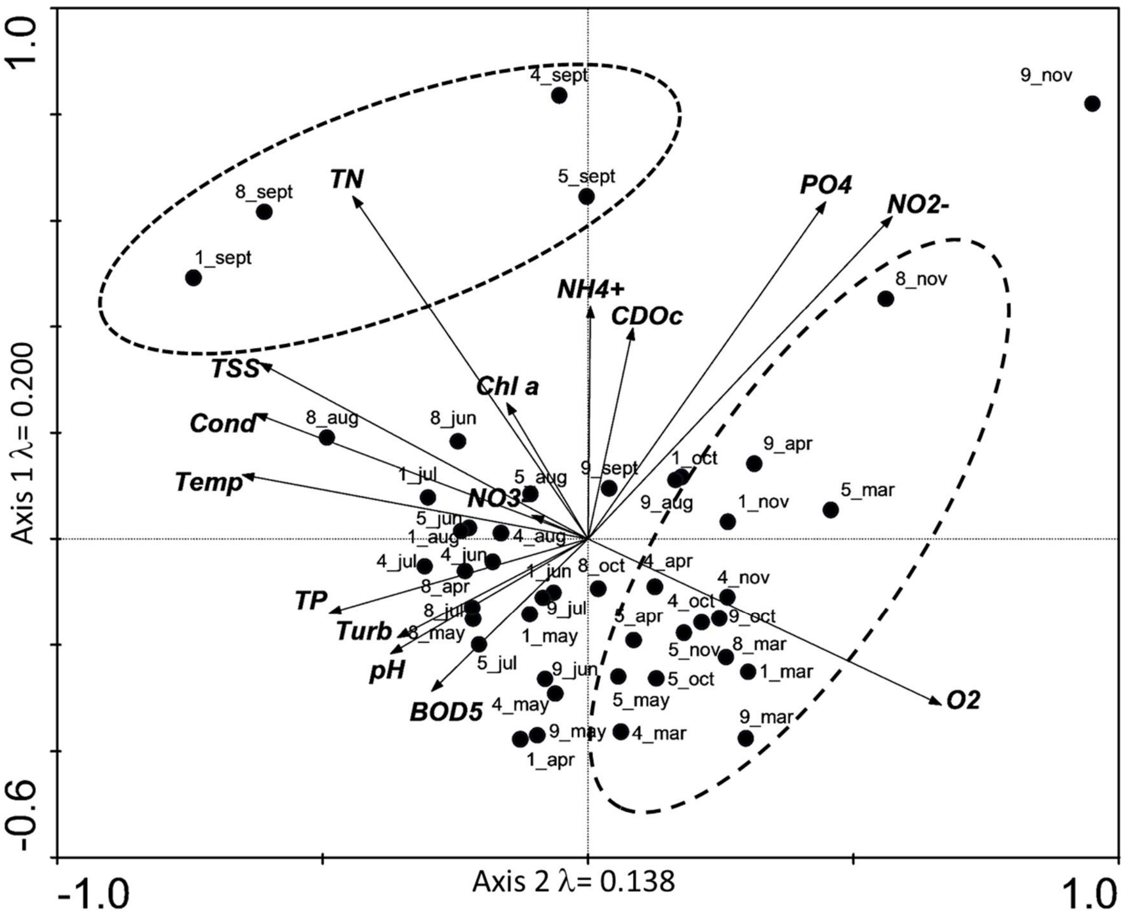


Fig. R1 – Graphical presentation of the PCA performed on the physical and chemical data obtained for each sampling site (site_month). TN – Total Nitrates, TSS – Total Suspended Solids, Cond – Conductivity, Temp – Temperature, TP – Total Phosphorus, Turb – Turbidity, Chl a – Concentration of chlorophyll *a*, O2 – Dissolved O_2 (mg/L), NO3 – Nitrates, NO2- - Nitrites, PO4 – Phosphates, NH4+ - Ammonium. --- stands for highlight the proximity of sites/seasons

Considering the distribution of the sites in figure R1, it is possible to verify that they are grouped according to a seasonal variation. Most of the samples from cooler months - March, April, October and November – were mostly grouped in the fourth quadrant (see highlights in Fig. R1), clearly influenced by high concentrations of dissolved O_2. The ability of water to incorporate O_2 increases as the temperature drops, so this segregation was expected. The warmer months are concentrated in the centre of the graphic, except for September (see highlights in Fig. R1), which has four of the sampling sites dragged away from the rest by high concentrations of total nitrates. This might be explained by the weather conditions during that month. Higher rainfall and wind caused high sediments resuspension in the water, as well as higher leaching of the surrounding fields. This may have increased the concentration of total nitrates and TSS in the water of the reservoirs of Venda Nova, Alto Rabagão and Alto Cávado. On the other hand, Paradela is, as already mentioned, very isolated from anthropogenic disturbance, so the soils around the reservoir are least susceptible to have high concentrations of contaminants (rich in nitrates), which could be washed by the rain into the reservoir. This explains why the point 9_sept (Paradela) is located away from the other four in the graphic. In November, high values of nitrites and phosphorus were registered for Paradela reservoir, which caused the point representing 9_nov so isolate from the others.

In the Table R1, are presented the range of values for physical and chemical elements proposed in WFD obtained in the sampling events. Regarding the comparison to the maximum thresholds values stablished for the "Good Ecological Potential" (GEP) for Northern Reservoirs.

Table R1 – Comparison between the maximum limits for physical and chemical parameters for Good Ecological Potential in northern reservoirs stablished by WFD and the values obtained in each sampling site. Bold values stand for outside the maximum limits established.

Parameters	Limit to GEP **Northern Reservoirs**	Observed Venda Nova **Site 1**	Alto Rabagão **Site 4**	Alto Rabagão **Site 5**	Alto Cávado **Site 8**	Paradela **Site 9**
Dissolved oxygen[(a)]	≥5 mg O_2/L	6.21±2.36	6.19±1.66	6.56±1.95	6.29±2.33	6.83±2.27
Oxygen saturation rate[(a)]	Between 60% and 120%	**38.6** -103.8	**42.2** – 86.9	**40.2** – 96.3	**31.5** – 88.9	**41.2** – 103.5
pH[(a)]	Between 6 and 9	7.56±1.11	7.53±0.87	7.64±0.71	7.77±1.47	7.45±0.76
Nitrates[(b)]	≤25 mg NO_3/L	0.98	1.34	1.03	1.58	1.23
Total Phosphorus[(b)]	≤0.05 mg P/L	0.039	0.034	0.023	0.05	0.027

(a) 80% of the samples if the sampling events are monthly or superior
(b) Annual average

For the dissolved oxygen parameter in mg/L, all the sampling sites had an annual average above the minimum value of 5 mg/L, and more than the 80% of the monthly values required for the classification of Good Ecological Potential were also above the minimum. Concerning the oxygen saturation rate (%), none of the sampling sites complied with the required 80% of the samples above 60% of oxygen saturation, although none have passed the 120% maximum limit. The lower values for all the sampling sites were registered during the months of June and July, when water reached higher temperatures. This situation can be explained by the fact that, as mentioned before, the water has lower ability to incorporate O_2 in higher temperatures.

In the analysis of the pH parameter, all of the five sampling sites meet the criterion that more than 80% of the monthly samples had pH in the 6 to 9 range required for the Good Ecological Potential. The pH values were very consistent through the entire sampled year in all the studied reservoirs.

Although some considerable variations occurred in the concentrations of nitrates during the sampling period, all the sampling sites had the required annual average of concentrations under the maximum limit of 25 mg/L. Similarly to the nitrates content, the concentrations of total phosphorus were very low for all the sampling sites through the entire year. The annual average was under the maximum limit stipulated for the good ecological potential in all the reservoirs.

Trophic State Index

The figure R1 presents the Trophic State Index (TSI) in the five sampling sites, using the concentrations of chlorophyll *a.* According to the scale defined by Carlson (1977), the variation of TSI for each site along the sampling period were classified by all water classifications: oligotrophic, mesotrophic, eutrophic and hypertrophic (Fig. R2).

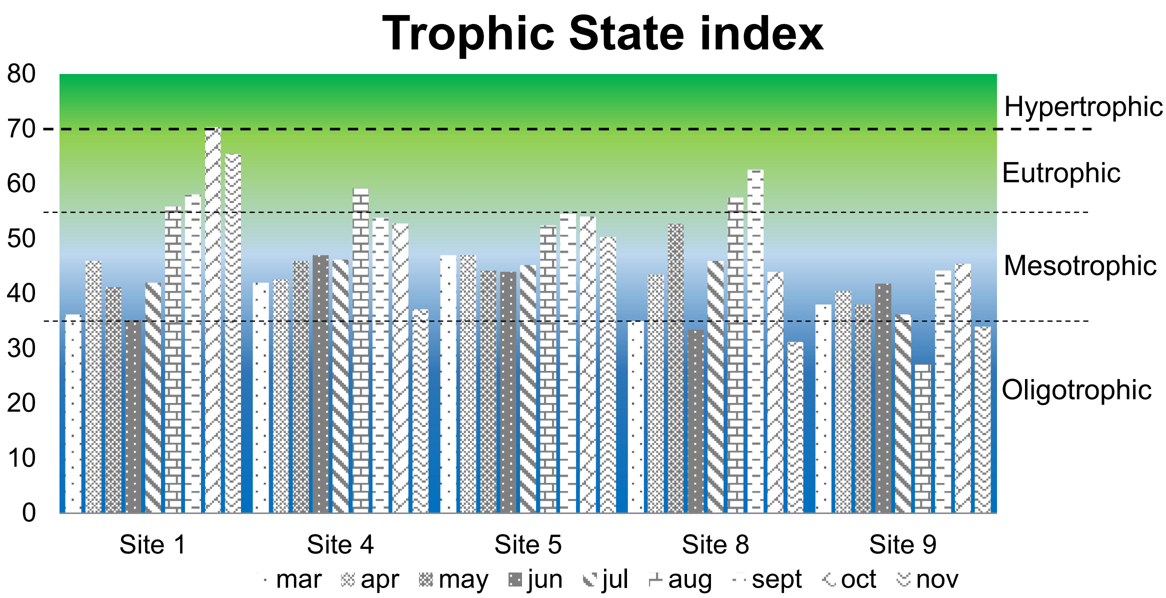
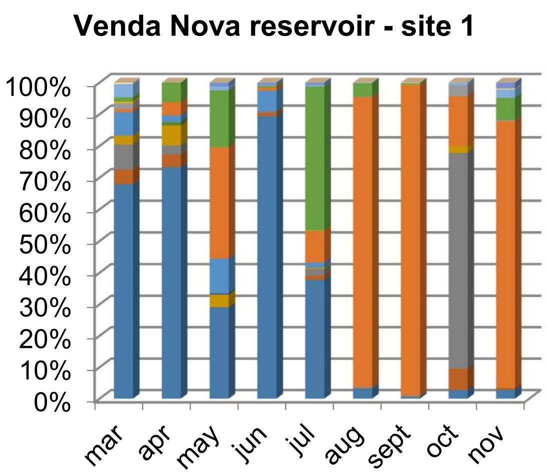


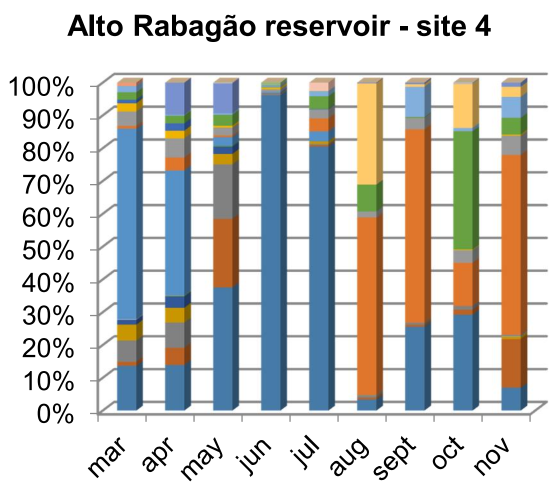
Fig. R2 – TSI obtained along the sampling period (March to November), for all sampling sites, classified according to the scale defined by Carlson (1977): oligotrophic, mesotrophic, eutrophic and hypertrophic.

Site 1 (Venda Nova reservoir) showed the most variations in the TSI through the sampling period. The TSI values ranged between the higher limit for oligotrophic classification, in June, and the lower limit for hypertrophic state, in October. In March, April, May and July, the reservoir was classified as mesotrophic, and as eutrophic in August, September and November. This high variation in TSI along the sampling period might be due to the great variations in the water level registered during the year of the study (2014). Both sites from Alto Rabagão reservoir, site 4 and site 5, showed relatively low variations in the TSI through the year, probably due to the great dimensions of this reservoir. Between the two of them, site 4 showed the higher variation, probably due to the proximity of the input channel of water from Alto Cávado's reservoir. In site 4, the classification ranged between mesotrophic in most of the months and eutrophic in August. In site 5, all the values obtained were under the mesotrophic classification. The sampling site 8 (Alto Cávado reservoir), as Venda Nova, showed high variations through the year. The values variated between oligotrophic, in June, and eutrophic in August and September, and the remaining months being classified as mesotrophic. Sampling site 9 (Paradela reservoir) was the reservoir that showed least variations in trophic state through the year. It was also the reservoir that showed the lower values for TSI, all comprehended between oligotrophic, in August and November, and mesotrophic in the remaining months.

Dynamics of the zooplankton communities

The data obtained from zooplankton samples was converted in proportion and exposed in a graphical presentation (Figure R3) to better understand the population dynamics for each sampling site during the studied period. In almost all of the sampling sites, it is possible to observe switches in the communities of cladocerans and copepods. Also, almost sampling site show higher species richness between March and July.





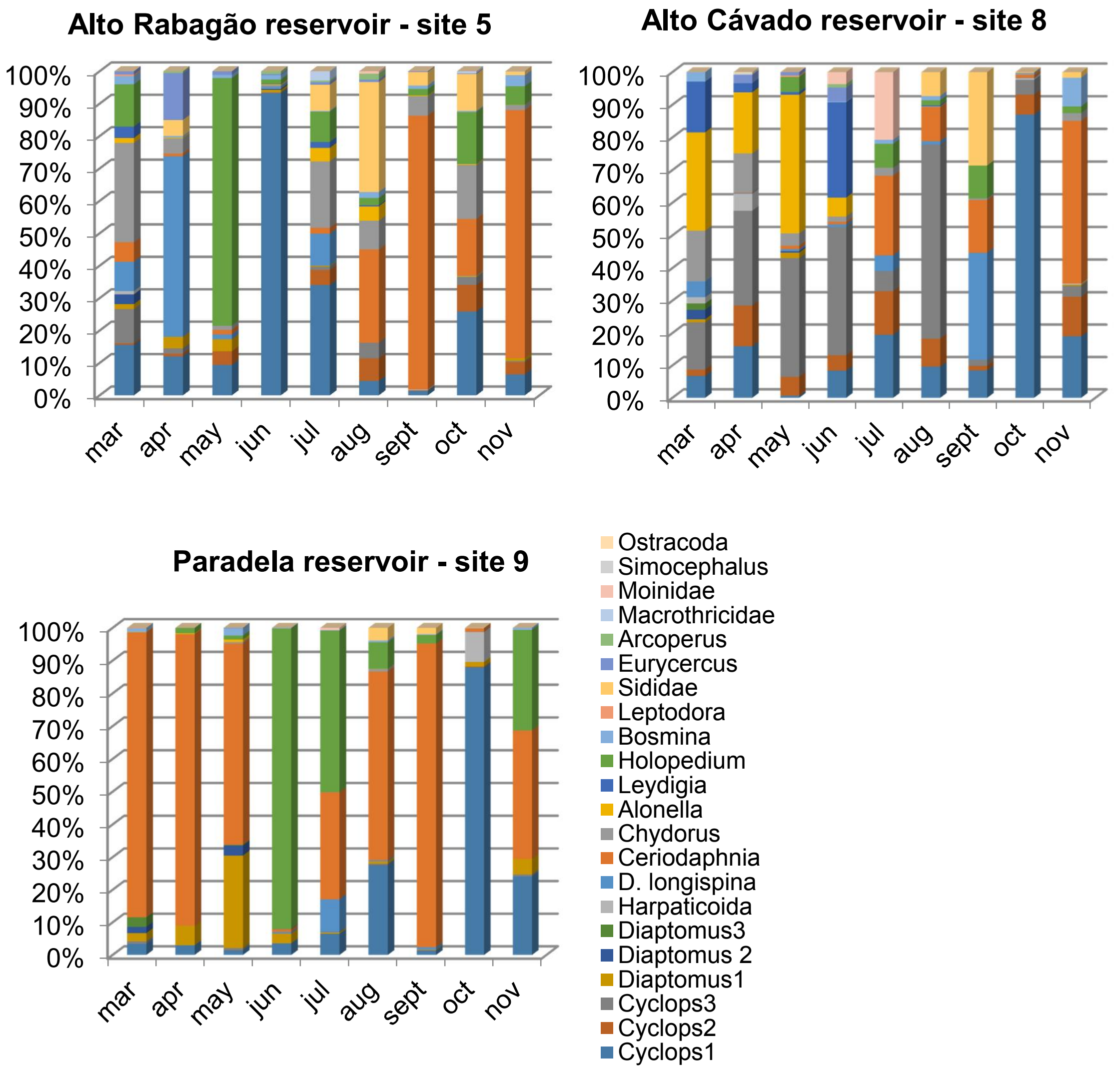


Fig. R3 – Graphical presentation of the dynamics and composition of zooplanktonic communities from Venda Nova, Alto Rabagão, Alto Cávado and Paradela along the sampling period. Each colour represents a different species of zooplankton.

Samples from Venda Nova (Fig. R3), cyclopoids, *Ceriodaphnia* sp. and *Holopedium* sp. were the most abundant organisms found through the sampling period. In the first two months, it is possible to observe a dominance of copepods, more specifically cyclopoids, in the zooplanktonic community, representing almost 80% of the community's organisms. In May, a considerable growth of cladocerans populations was verified, particularly for *Ceriodaphnia* sp., which were the most abundant organisms during that month. Considering that in May a drastic reduction of the water level occurred, due to works in the dam, which may have caused the switch in the community's composition and dominances. In June, the cyclopoids were the dominant species and a massive growth of the population was verified. This may be related to the rising of the temperature of the water in this month. In July, the cladocerans populations began to grow and occur the appearance of the *Holopedium* sp., a species that develops preferentially during the summer season. Cladocerans were completely dominate through August and September. In October the water level rose again to the normal volume, which may have caused another interruption on the natural seasonal dynamics of the zooplanktonic community. In the last month, once again the *Ceriodaphnia* sp. were the dominant species in the reservoir.

The two sampling sites from Alto Rabagão, site 4 and 5, had similar communities' dynamics and species composition (Fig. R3). During most of the year, cladocerans are dominants in the zooplankton's communities, and the most common organisms for both sites were cyclops and *Ceriodaphnia* sp.. In site 4, *Daphnia longispina* is the dominant species during March and April. This species develops during winter and spring, preferably in unpolluted waters. In May, the cyclopoids populations were the dominant species and kept the high representation during June, when they represented almost 100% of the zooplankton's community. In July, a small decay of the cyclopoids was observed, however still represent 80% of the organisms registed. In August, the cladocerans populations grew and dominate during the remaining sampling months, particularly with *Ceriodaphnia* sp., which were dominant during August, September and November. Site 5 had more presence of *Chydorus* sp., occurring in high quantities during March, July and October. The first three months of the sampling period, March, April and May, showed high dominance of the cladocerans populations, particularly *Chydorus* sp., *Daphnia longispina* and *Holopedium* sp., respectively. In June, cyclops was the dominant group, representing approximately 93% of the organisms collected. In July, the cladoceran populations returned to being dominant, until the final of the sampling period. *Ceriodaphnia* sp. showed high representativeness during September and November.

Alto Cávado's reservoir showed a different species composition and higher diversity when compared to the other three reservoirs (Fig. R3). It is possible to verify a higher presence of margin species, such as *Alonella* sp. and *Leydigia* sp.. Also, the species of cyclopoid commonly found in this site was different from the one identified for the other reservoirs. In March, cladocerans was the dominant species, with high representativeness of *Alonella* sp., *Chydorus* sp. and *Leydigia* sp. In April, May and June, the cladocerans and copepod communities were relatively balanced. In July, cladocerans were in greater number, however, this month showed high biodiversity in the zooplankton community's structure. In August copepods cyclopoids were the most represented group, while in September, a great number of *Daphnia longispina* and *Sididae* sp. was verified. In October, the zooplanktonic community was composed almost entirely by copepods cyclopoids. The population of cyclopoids declined in November, being replaced by *Ceriodaphnia* sp..

Paradela was the reservoir that showed lower species richness and lower switches in the zooplanktonic community (Fig. R3). Cladocerans were dominant for most of the year, particularly the populations of *Ceriodaphnia* sp. and *Holopedium* sp.. *Holopedium* species usually develops during the summer season, which explains the high representativeness of the population in June and July. Cyclopoids were only dominant during October, a month were a considerable rise of the water level was verified, cause by the high rainfall of September and October. This may have disturbed the community's stability and caused the dominance of cyclopoids species. It could also be cause by the end of a diapause period of this species.

Canonical Correspondence Analysis

The composition of zooplanktonic community and the physical and chemical data assessed from for each month for the five sampling sites were combined using a CCA, to understand what were the correlations between them. The first two axes of the CCA were able to explain a total variance of 50.4% of the distribution and similarities in the zooplanktonic species for each sampling site. A graphical representation of the CCA was used to helping the results interpretation, presented in the figure R4.

In contrast to the observed in the PCA for the environmental parameters, in CCA representation the points are distributed according to the reservoir they belong and not by seasonality. In the centre of the graphic it is possible to identify most of the points correspondent to the site 4 and site 5. These two sites are located in the same reservoir, so it is expectable observe a similar species composition. Samples from Paradela (sampling site 9) are also mostly located together, in the fourth quadrant of the graphic (see highlisht in Fig.R4), more influenced by the occurrence of *Holopedium* sp. Considering the stability observed in the dynamics of the zooplankton's community from this reservoir (Fig.R3), it was expected a similar distribution for the different sampled months. Points corresponding to Alto Cávado samples (8) also showed a distinct distribution from the remaining sites, mostly located in the second quadrant (see highlight in Fig. R4). These are clearly most influenced by margin species such as *Leydigia* sp., *Alonella* sp. and *Eurycercus* sp.. In contrast, sampling site 1 (Venda Nova reservoir) was the one that showed lower consistency in the distribution. This was possibly caused by the great variations in the water level of the reservoir observed during the sampling period.

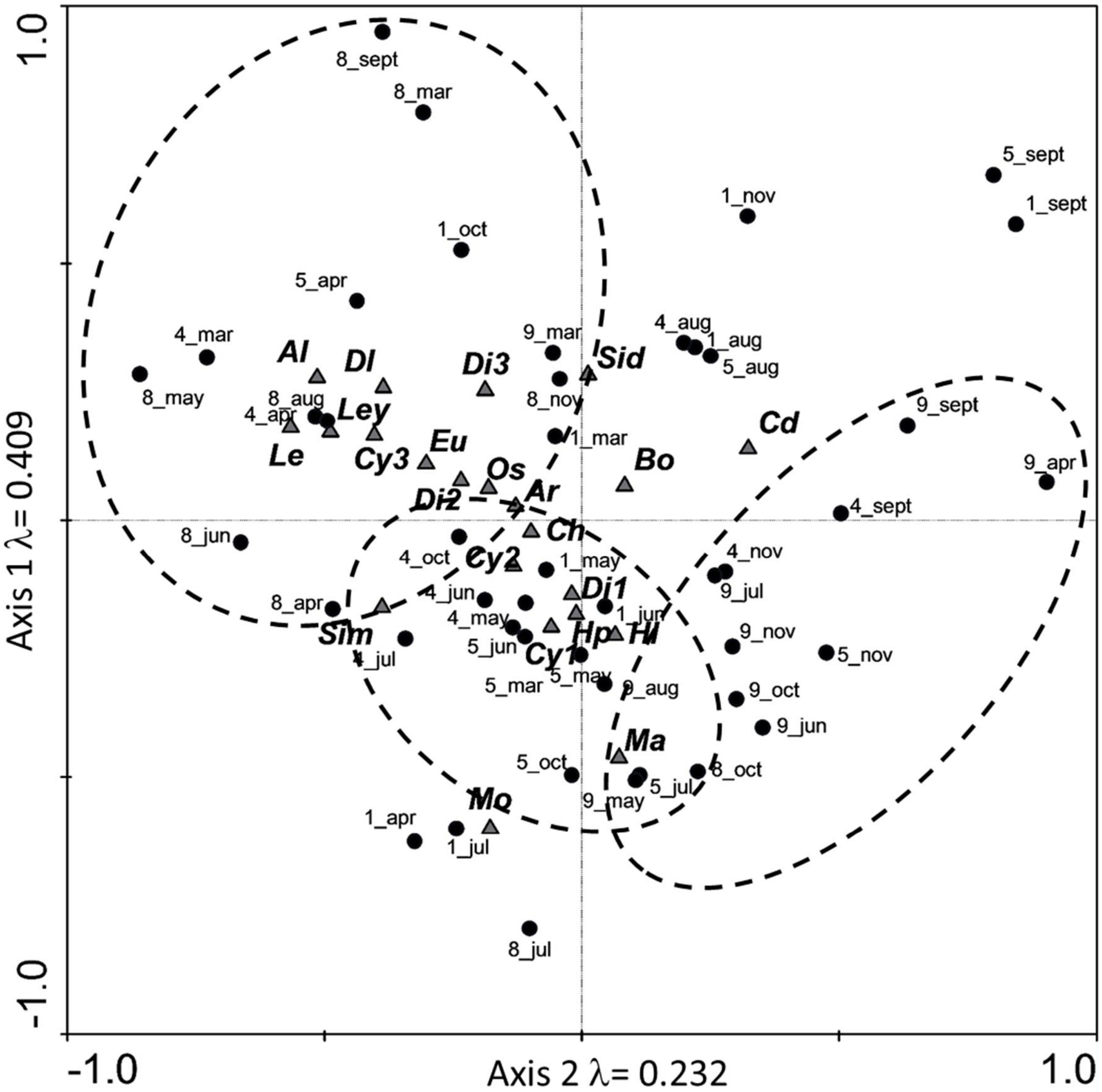


Fig. R4 – Graphical presentation of the CCA performed using both zooplankton's community and physical and chemical data obtained for each sample. Cy1 – Cyclopoid, Cy2 – Cyclopoid, Cy3 – Cyclopoid, Di1 – *Diaptomus sp.,* Di2 – *Diaptomus sp.,* Di3 – *Diaptomus sp.*, Al – *Alonella sp.*, Dl – *Daphnia longispina*, Le – *Leptodora sp.*, Ley – *Leydigia sp.*, Eu – *Eurycercus sp.*, Os – *Ostracoda sp.*, Ar – *Arcoperus sp.,* Ch – *Chydorus sp.*, Sid – *Sididae sp.*, Bo – *Bosmina sp.*, Cd- *Ceriodaphnia sp.*, Hp – Harpaticoida, Mo – *Moina sp.*, Sim – *Simocephalus sp.*, Hl – *Holopedium sp.,* Ma – Macrothricidae. ---- stands for highlight the proximity of sites/seasons

Discussion

Discussion

Several studies have shown the relationships between the land-use and the quality of the water in the respective watershed (Lee et al., 2009; Pan et al., 2004; Smith et al., 1999b). Waterbodies surrounded by agricultural fields and croplands are subjected to larger inflows of nutrients, resultant from the application of fertilizers and manures on the soils (Navarro et al., 2009b; Turner and Rabalais, 2003). Wildfires and forest management can also affect the water quality, compromising the ability of the soil to retain sediments and degrade nutrients (Santos et al., 2015). The reservoirs chosen for this project are located in a rural area, isolated and subjected to very low anthropogenic disturbance and surrounded by vast forestall and natural areas and small agricultural holdings (Cabecinha et al., 2009a; Cabecinha et al., 2009b; Santos et al., 2015). Therefore they were expected to have good water quality and show low disturbance, besides those caused by the dams regime and the leaching from the surrounding areas. Through multivariate analysis of the environmental data, it was possible to observe that water mass quality varied according to a seasonal pattern. The relatively homogeneous variation of all the sampling sites along the sampling period showed that the ecosystems were very similar in the variations of the water quality during that year. One of the most influent variables was dissolved O_2 (in mg/L), which was inversely proportional to the raising of the temperature of the water. This was expected, considering that lower concentrations of O_2 are usually registered during warmer months (Celekli and Öztürk, 2014; Czerniawski and Domagała, 2010). The high influence of a parameter that varies according to its natural pattern shows that the ecosystems were little disturbed by external factors. The relatively isolate location of the chosen reservoirs explains the lack of anthropogenic disturbances in the water quality. In addition to this, considering the presence of agricultural fields in the watershed, it was also expected some input of nutrients from leaching. The peak in concentration of total nitrates that was verified in September occurred simultaneously in all reservoirs, with exception of Paradela reservoir (the most isolated and undisturbed of the chosen reservoirs), where high variations in the concentration of this nutrient were not verified. The month of September was also marked by the increase of the rainfall in that area. According to Chen et al. (2002), rainfall is a major enhancer of the nitrogen load in the aquatic systems without a specific pollutant source, through surface runoff and underground flow. This allows us to infer that the increase of the concentration was caused by leaching from the surrounding areas and not by a specific contamination source. However, the physical and chemical data collected were only able to explain 33.8% of the variations. To increase this value, more environmental parameters should be assessed in further work, such as the presence of specific pollutants, the variation in the water level, and precipitation.

The comparison between the physical and chemical values in the studied reservoirs and the limits established by WFD for the Northern Mediterranean reservoirs for the Good Ecological Potential allowed us to include all of them within this classification. The data available in SNIRH database shows that, in the past ten years, these reservoirs have all obtained annual classifications within Good Ecological Potential in most of the years. However, reservoirs sporadically obtained the classification of Moderate Ecological Potential due to unusual leaching of nutrients and microbial content. Venda Nova was the only that obtained a classification of Bad during the last 10 years, due to an unusually high concentration of phosphates. According to Cabecinha et al. (2009a), these reservoirs can be considered as reference for Good Ecological Potential, based on environmental data assessed by the Laboratory of Environmental and Applied Chemistry (LABELEC). Comparing to the data obtained in their sampling period (between 1996 and 2004) with our results for the pH, dissolved oxygen and nitrates, it is possible to verify that they are very similar. This shows that the quality of the water of these reservoirs has kept good and stable in the past years and that our results are in concordance with this tendency. Although, some slight variations in the nutrient concentrations were verified during our sampling period, probably caused by leaching of the surrounding areas to the reservoirs, given that all the reservoirs, except for Paradela reservoir, are surrounded by agricultural fields, which means the soils are probably contaminated with high concentration of nitrates and phosphorus. The fact that Paradela was the reservoir that showed lower variations in the concentration of nitrates supports this hypothesis, since it is the least susceptible to contamination by leaching among all the sampling sites.

The analysis of the physical and chemical parameters on their own and under the perspective of the WFD indicates that all the reservoirs were very stable along the sampled year and there were no significant variations in the water quality, nor great disturbances caused by the dam use and variations in the reservoirs. However, considering the results obtained with the TSI and the zooplankton communities it is possible to infer that some changes occurred in the structure of the ecosystem.

Although the environmental parameters appear to have had a reasonably homogenous variations, the information provided by the biological parameters assessed may show some differences. Many authors have studied the importance using biological elements do evaluate water quality and the ecological status on aquatic systems (Cabecinha et al., 2009a; Martinez-Haro et al., 2015a). Elements such as phytoplankton have great sensibility to alterations on the nutrient concentrations in the water (Schindler, 1977) and, therefore, are widely used as indicators of water quality. Considering the distinct variations of the TSI observed on the reservoirs, it is possible to assume that they suffered from different pressures along the year. Venda Nova was the reservoir that suffered higher variations in the structure of the reservoir, due to great decrease of the water level in May caused by works in the dam and the rose to normal levels (Fig. MM4) in October. The analysis of TSI for this reservoir reflects the impact of this variations in the ecosystem. The flood of the soils can cause an increase of the concentration of nutrients in the water body, such as nitrates and phosphorus, responsible for the eutrophication processes (McCartney et al., 2000; Navarro et al., 2009b). This explains the peak on the TSI values observed in October in Venda Nova reservoir, when the rise of the water level may have dissolved nutrients present in the exposed soils and increased its concentration in the water, leading the ecosystem to the state of hypertrophy. Alto Cávado also showed high variation in TSI across the year. Considering the reduced dimensions of this reservoir and according to Padisák et al. (2003), smaller reservoirs are more vulnerable to changes caused by climatic variations and human activities, are therefore subjected to more variations in the phytoplankton community. The instability verified on the trophic state on this reservoir may be related to its size, considering the relatively small dimensions of Alto Cávado.

The load of nutrients and the trophic state of an ecosystem are key factors to determine the structure of zooplankton communities (Barnett and Beisner, 2007; Jensen et al., 2013; Jeppesen et al., 2011b; O'Brien et al., 2004). This relation between trophic state and the zooplankton could be observed through the comparison of our results for TSI and the dynamics of zooplankton communities. Although some of the changes observed in the structure of the communities might be associated with the biology of the species, the high sensitivity of zooplankton to the environmental conditions is also responsible for many of the changes that occurred in the structure of the communities (O'Brien et al., 2004). The populations of zooplankton respond to short term changes in the environment, such as the alteration of nutrient loading in the ecosystem (Jeppesen et al., 2011a). When compared the TSI results, many of the switch in the species representativeness in zooplankton communities coincide with changes in the trophic status. During August in Venda Nova reservoir a considerable increase in the trophic state was verified and it was coincident with a major switch in the dominance in the zooplanktonic community. The *Holopedoium* sp. population, which are highly associated to environments with low trophic states (Jensen et al., 2013), was almost supressed, and *Ceriodaphnia* sp. became dominate. The same phenomenon was observed in the same month for sampling sites 4 and 5 (more significantly in 4, where the variations of TSI were also more intense). The increase in trophic state can be associated to an increase of small bodied cladocerans, considering that they are more efficient bacterial feeders (Jensen et al., 2013), and species more tolerant to eutrophication such as *Ceriodaphnia* sp. (Azevêdo et al., 2015). Amoros (1984) also described most of the *Ceriodaphnia* sp. as being very tolerant to high trophic status, thus the high dominances of these organisms observed in months when high trophic values where registered are expected. In almost all of the sampling sites, it is possible to observe switches in the communities of cladocerans and copepods across the sampled period. Copepods are usually more representative during spring months (Nogueira, 2001). This could be observed in our results where, for most of the sampling sites, copepods where more abundant until July. Then, coincidently with an increase of TSI values for most of the sampling sites, the communities were manly composed by cladocerans. The increase of primary production may cause the growth of filtering species of the zooplankton (Hessen et al., 2006), such as cladocerans.

Particularly in Venda Nova reservoir, it was also possible to observe alterations in the zooplanktonic community coinciding with major alterations in the water level registered in May (water level drop) and October (water level rise). Major fluctuations in the water level may cause disturbances in the aquatic ecosystems. The increase of suspended particles resulting from reservoir emptying can result in an increment of nutrient and chlorophyll *a* concentrations (Geraldes and Boavida, 2007). In our results for Venda Nova reservoir, *Ceriodaphnia* sp. and cyclopoids were the two most representative groups. This can be a reflection of the reservoir instability because, according to Geraldes and Boavida (2007), *Ceriodaphnia* sp. and a cyclopoid species are organisms commonly dominate in zooplanktonic communities from reservoirs that are subjected to this sort of disturbance. Additionally, the species composition observed in this reservoir was very unstable. The CCA performed on the zooplankton community data collected showed that Venda Nova had the lower consistency in the species composition and dominances, considering the very heterogeneous distribution of the points in the graphical presentation. The drop on species richness observed in May can also be related to the sudden alteration on the ecosystem stability and, consequently, on the species composition (Geraldes and Boavida, 2007).

Zooplankton community species richness is also related to the size of the reservoir. Usually, species richness increases with the increase of ecosystem area (O'Brien et al., 2004). In our results, the reservoirs that showed higher species richness were Alto Rabagão and Alto Cávado. As mentioned in the description of the studied reservoirs, Alto Rabagão was the greatest of the four, so it is possible for us to assume the correlation between the high species variability and the size of the reservoir. On the other hand, Alto Cávado is the smallest and shallower of the sampling sites but it also showed a very high species richness. When analysing the species found in the samples from Alto Cávado, many were littoral species, such as *Leydigia* sp. and *Alonella* sp. (Alonso, 1996), and could only be found in samples from this reservoir. This is observations are probably related to the local where the samples were collected (near the shore) rather than to the size of the reservoir. The low depth of the reservoir and the fact that our samples were collected very close to the margins and on areas with high density of submerged vegetation and macrophytes (Hessen et al., 2006). This allowed for both pelagic and littoral species to be captured by the net. This can also be verified in the CCA, were the points representing samples from Alto Cávado are more homogenously associated with littoral species. Species richness and replacement observed in Alto Cávado can also be related to alterations in the trophic state. The increase of the trophic state can cause littoral populations to decrease and the growth of pelagic species. The decrease of light penetration due to eutrophication process causes the death of submerged vegetation and the deterioration of the littoral habitat, while it also increases the food particles available in the water column for the filter feeding organisms (Jensen et al., 2013). In our results the population dynamics for Alto Cávado reservoir, the littoral species decreased in the month of July, coinciding it an increase of the trophic status, being replaced for pelagic species, such as *Ceriodaphnia* sp..

The Paradela reservoir was the one that had higher stability of the zooplanktonic community. The high presence of *Holopedium* sp., a species very intolerant to eutrophication phenomenon (Jensen et al., 2013), and the small shifts on both species composition and TSI values along the sampling period allows us to infer that this reservoir suffered from very low nutrient input and external disturbances. Even through the CCA analysis, all the points correspondent to this reservoir had a very consistent distribution.

As observed by some other authors (Caroni and Irvine, 2010; Jeppesen et al., 2011a), zooplankton provides a very complete image of alterations occurred on the ecosystem and its structure and functionality, in contrast to the information provided by the environmental data.

Conclusion

Conclusion

In the light of the results obtained in this study and a literature review about this issue, physical and chemical data gave us the image of high quality and stability for all the reservoirs studied, even according to the parameters stablished by the WFD. Despite that, when further analysis of the zooplanktonic community was performed, it was possible to observed that all of the reservoirs where very different in terms of community structure and had different dynamics along the sampling period. This analysis gave us a much deeper understanding on the alterations occurred on the reservoirs. While environmental data can only provide isolated information of a static moment (when the samples are collected), zooplankton dynamics reflect the evolution of the ecosystem during the same period and the combined effect of different parameters that affected the studied reservoirs.

Considering this, it is possible to perceive that the high sensibility of the zooplankton's responses to ecosystem changes can be used as a success tool to evaluate water quality. Although environmental parameters are currently the most developed component to the analysis proposed by WFD for reservoirs, in many cases they may not be enough to fully assess the ecosystem's function. The inclusion of zooplankton as a biologic elements in WFD would provide a much deeper understanding of the ecosystems functionality and stability.

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Annexes

Annex I – Physical and Chemical Data

	pH	Cond	O2 mg/L	Temp	BOD_5	TSS	Chl a	Turb	CDOc	TN	TP	NH_4^+	NO_2^-	NO_3^-	PO_4
1_mar	6,85	18,9	7,62	11	1,37	2,877	1,78	0,004	2,3	0,1	0,01	0,37	0,004	0,10	0,29
1_abr	6,89	19,9	5,94	15,5	2,64	7,12	4,81	0,03	1,84	0,1	0,04	0,19	0,030	0,10	0,15
1_mai	7,35	20,9	2,21	17,2	1,78	9,72	2,94	0,002	1,15	0,1	0,03	0,01	0,004	0,10	0,13
1_jun	8,47	21,7	5,906	18,7	1,32	9,12	1,6	0,008	5,75	0,886	0,06	0,04	0,080	0,10	0,49
1_jul	6,85	23	4,53	23	1,79	24,23	3,2	0,006	5,98	0,1	0,04	0,01	0,046	5,60	0,49
1_ago	10,09	27,2	5,74	21	0,56	13,23	13,17	0,006	5,98	2,215	0,01	0,10	0,004	0,10	0,40
1_set	7,79	27	5,04	17,2	1,46	42,63	16,55	0,008	5,98	13,29	0,11	0,07	0,037	2,50	0,11
1_out	6,786	18,4	10,19	15,5	1,43	9,62	57,67	0,008	9,89	0,1	0,05	0,16	0,084	0,10	0,98
1_nov	6,92	20,2	8,7	11,5	1,44	6,65	34,82	0,003	12,42	0,1	0,00	0,54	0,004	0,10	0,18
4_mar	7,03	21,7	8,11	10,7	1,46	5,52	3,2	0,006	2,76	0,1	0,12	0,01	0,004	0,10	0,05
4_abr	7,02	22,4	6,11	13,9	0,84	6,88	3,39	0,01	2,99	0,1	0,03	0,21	0,057	0,96	0,71
4_mai	8,07	21,5	6,482	16,9	2,08	9,02	4,81	0,007	1,61	0,1	0,03	0,01	0,004	3,20	0,02
4_jun	8,26	21,6	4,882	21,9	0,47	13,22	5,34	0,017	6,44	0,1	0,03	0,04	0,042	0,10	0,41
4_jul	8,26	25,2	5,18	20,8	2,39	15,86	4,9	0,004	4,14	0,1	0,05	0,71	0,020	7,30	0,27
4_ago	8,95	21,6	4,8	20,6	0,57	13,002	18,48	0,006	6,21	1,329	0,02	0,03	0,004	0,10	0,16
4_set	6,53	22,2	3,81	19,1	1,38	16,42	10,68	0,003	4,14	7,088	0,01	4,17	0,170	0,10	1,08
4_out	7,026	21,5	8,32	16,6	0,95	6,32	9,61	0,002	2,3	0,1	0,01	0,01	0,029	0,10	0,27
4_nov	6,6	21,9	8,05	12,2	1,48	8,214	1,96	0,003	9,2	0,1	0,01	0,11	0,034	0,10	0,09
5_mar	7,245	21	8,87	10,8	0,99	8,15	5,34	0,005	2,3	0,1	0,02	0,01	0,357	0,10	0,22
5_abr	7,3	20,4	6,47	14,8	0,98	7,72	5,34	0,005	2,3	0,1	0,02	0,01	0,004	5,50	0,02
5_mai	7,56	21,4	6,546	16,7	1,92	6,92	4,01	0,001	1,38	0,1	0,02	0,01	0,004	0,10	0,12
5_jun	8,53	21,6	4,69	22,5	0,69	17,63	3,74	0,012	5,52	0,1	0,04	0,21	0,047	1,61	0,62
5_jul	8,7	21,3	5,96	22	1,75	14,19	4,45	0,004	2,76	0,1	0,05	0,07	0,004	0,10	0,20
5_ago	8,21	21,7	4,88	20,8	0,55	11,09	9,26	0,003	4,14	3,987	0,02	0,13	0,004	0,10	0,56
5_set	6,78	21,8	3,84	18,5	1,18	10,32	11,48	0,002	3,22	12,847	0,00	0,17	0,143	1,60	2,86
5_out	7,105	21	9,24	17	2,23	7,32	10,95	0,005	2,3	0,1	0,02	0,13	0,015	0,10	0,35
5_nov	6,95	21,8	8,55	12,1	2,32	8,157	7,48	0,007	11,04	0,1	0,02	0,07	0,023	0,10	0,11
8_mar	7,92	22,6	7,98	8,8	0,28	5,72	1,60	0,008	3,91	0,1	0,01	0,01	0,004	2,40	0,25
8_abr	6,81	24,2	5,33	16,6	1,22	22,42	3,74	0,006	4,37	0,1	0,09	0,04	0,004	0,10	0,20
8_mai	7,46	24,9	6,29	17,5	1,83	10,74	9,54	0,013	3,45	0,886	0,06	0,26	0,012	8,80	0,06
8_jun	8,83	25,5	3,858	25,1	0,35	12,52	1,34	0,01	3,91	0,886	0,07	0,20	0,028	0,10	3,14
8_jul	10,5	33,1	8,12	23,1	3,14	0,63	4,81	0,012	8,97	0,1	0,06	0,61	0,098	0,10	1,69
8_ago	9,1	39,1	4	22,9	0,93	15,53	15,58	0,009	10,35	2,215	0,03	0,22	0,004	0,10	0,11
8_set	5,93	44,1	3,05	17,5	1,98	12,63	26,17	0,013	12,88	13,733	0,08	0,10	0,032	0,10	0,70
8_out	6,71	27	8,93	15,3	2,23	9,75	3,92	0,014	17,02	0,1	0,04	0,19	0,004	0,10	0,25
8_nov	6,63	21,8	9,09	10,3	0,34	7,131	1,07	0,008	15,41	0,1	0,02	0,81	0,253	2,40	3,13
9_mar	7,65	13,6	11,47	10	0,66	10,72	2,14	0,002	1,61	0,1	0,10	0,19	0,004	0,10	0,19
9_abr	7,08	14,3	6,38	15,2	0,74	9,04	2,75	0,004	16,1	0,1	0,00	0,21	0,080	1,09	0,94
9_mai	7,81	14,9	5,714	19,3	2,07	8,68	2,14	0,018	0,69	0,1	0,02	0,01	0,004	2,00	0,04
9_jun	8,401	14,1	5,682	19,4	1,97	10,96	3,14	0,004	1,84	0,1	0,07	0,21	0,025	0,10	0,25
9_jul	8,725	14,7	5,95	22,2	0,71	13,914	1,78	0,004	3,68	0,1	0,04	0,53	0,004	2,40	0,24
9_ago	6,98	16	4,82	21,8	0,28	11,09	0,71	0,002	2,99	0,1	0,00	0,08	0,113	0,10	1,13

9_set	6,35	14,7	4,33	19,1	1,39	7,32	4,01	0,001	0,23	7,088	0,00	0,40	0,032	2,20	0,16
9_out	6,88	13,1	8,46	18,5	0,91	11,32	4,54	0,001	1,38	0,1	0,01	0,35	0,015	0,10	0,40
9_nov	7,18	11,9	8,62	13	0,65	6,21	1,42	0,001	8,74	0,1	0,00	0,09	0,536	3,00	7,41